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Ticks and tick-borne diseases in the city : role of landscape connectivity and green space characteristics in a metropolitan area

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1 **Ticks and tick-borne diseases in the city: role of landscape**  
2 **connectivity and green space characteristics in a metropolitan**  
3 **area**

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## 21 Abstract

22 Green spaces in the city are important for human wellbeing, ~~ecosystem services and~~  
23 ~~biodiversity~~, but are also zones in which humans ~~and domesticated animals~~ can become infected  
24 with zoonotic diseases. Therefore, there is a need to understand how infection risk is related to  
25 green space characteristics, wildlife communities and connectivity with rural areas hosting  
26 reservoir populations of hosts. Our ~~basic~~ hypothesis is that ~~the presence and abundance of~~  
27 wildlife hosts in urban green spaces, and thereby the prevalence of questing ticks and their  
28 Lyme disease causing pathogens (*Borrelia burgdorferi* s.l.), can be partly predicted based on  
29 green space characteristics (~~size, habitat, urbanization and cultivation~~) as well as measures of  
30 connectivity to known source areas. We sampled ticks ~~and their pathogens~~ in twenty-two green  
31 spaces ~~during Spring (2014 and 2016) and Autumn 2016~~, located along an urbanization gradient  
32 in Antwerp (Belgium). ~~More than 18,000 m<sup>2</sup> was sampled, with tick densities ranging from 0~~  
33 ~~to 386 individuals/100 m<sup>2</sup>~~. We estimated ~~landscape~~ connectivity using the least-cost algorithm  
34 as either the cost distance to the nearest green space, or to a known population of roe deer  
35 (*Capreolus capreolus*), known to be an important tick propagation host ~~for ticks~~. Both  
36 connectivity measures turned out to be ~~highly~~ correlated, reflecting a gradient in green space  
37 isolation from the periphery to the urban center. ~~In 87 % of plots where ticks were trapped, at~~  
38 ~~least one *Borrelia*-infected tick was found. The overall *Borrelia*-prevalence in nymphs was 17.8~~  
39 ~~%, in adults 32.6 %~~. ~~We found that~~ D-density of infected ticks decreased with urbanization and  
40 increased with connectivity. Nymphs in larger green spaces were more likely to be infected  
41 ~~with *Borrelia*~~. While ~~tick~~-density and infection prevalence for adults increased with the amount  
42 of neighboring agricultural land, the larval density ~~of larvae~~ and nymphal infection prevalence  
43 ~~in nymphs~~ decreased. Interestingly, the proportion of *Borrelia* genospecies associated with  
44 birds or mammals was comparable in rural and (sub)urban areas (bird/mammal: 0.38),

45 suggesting that even in small green spaces ~~with low tick densities,~~ *Borrelia* infections can  
46 persist in local host populations.

47

48 **Key terms:** landscape connectivity, urban, *Ixodes ricinus*, *Borrelia burgdorferi* s.l.

## 49 **1. Introduction**

50 Urbanization is one of the most drastic and widespread manifestations of human-driven  
51 environmental change. By 2050, more than 70% of the world population is expected to live in  
52 urban areas (Zipperer and Pickett, 2012). To promote biodiversity, ecosystem functioning and  
53 human well-being, there is now an increasing effort to maintain urban green spaces and improve  
54 their ecological connectivity (e.g. Haase et al., 2014). Green infrastructures are promoted as  
55 multifunctional “nature-based solutions” for a wide range of services including water  
56 management, air quality, temperature, recreation and ecosystem services directly related to  
57 biodiversity such as pollination (Hansen and Pauleit, 2014; Perini and Sabbion, 2016).  
58 However, urban greening also increases the risk of human exposure to animal-associated  
59 parasites and pathogens (e.g. Braks et al., 2016; Mackenstedt et al., 2015; Rizzoli et al., 2014).  
60 Hence, there is an urgent need for more research on the effect of ecological connectivity and  
61 urban wildlife on human exposure to zoonotic infections.

62 Hard ticks (Ixodidae) are important vectors of human and animal pathogens in the temperate  
63 climate zone, notably of Lyme borrelioses, the most prominent among tick-borne diseases  
64 (TBD). In Europe, the main tick vector is *Ixodes ricinus* (Gray, 1998), which is increasingly  
65 observed in urbanized environments with pathogen prevalences that may be as high as those in  
66 rural sites (Rizzoli et al., 2014; Uspensky, 2014). Ixodid ticks feed once in each life stage on a  
67 different host individual, usually a different species due to the tick’s exophilic biology. Since *I.*  
68 *ricinus* is a generalist parasite, the majority of wild as well as domestic animals present in urban

69 environments can potentially serve as tick-maintenance hosts, and in turn get exposed to a  
70 variety of pathogens to which they may be susceptible or (become) resistant. Especially in  
71 suburban areas, medium to large sized mammals (e.g. fox, deer, hedgehogs) occur, which are  
72 *I. ricinus* end-hosts, and therefore these areas may act as population sources for *I. ricinus* (e.g.  
73 Rizzoli et al., 2014). Mobile hosts such as birds may also introduce ticks to locations where  
74 they may pose a risk to humans, even if no suitable hosts are available to complete a full  
75 lifecycle.

76 Ticks generally acquire Lyme spirochetes through horizontal transmission from an infected  
77 host, or when co-feeding with an infected tick (Gern and Rais, 1996; Voordouw, 2015). Hosts  
78 differ in their suitability to feed different life-stages of ticks, but also in their capacity to transmit  
79 different *Borrelia burgdorferi* genospecies (Kilpatrick et al., 2017; Piesman and Gern, 2004).  
80 Particular *Borrelia* genospecies are associated with specific host types due to differences in host  
81 serum sensitivity (Kurtenbach et al., 1998). In Europe, genospecies are associated with different  
82 hosts and show different clinical manifestations in humans: e.g. *B. garinii* is associated with  
83 neuroborreliosis and typically acquired by ticks feeding on birds, while *B. afzelii* causes  
84 dermatological symptoms and is acquired from small mammals (see references in Braks et al.,  
85 2016). Some host species such as deer are not competent in hosting any *Borrelia* genospecies  
86 (LoGiudice et al., 2003; Matuschka et al., 1992).

87 Thus, local tick abundances and *Borrelia* prevalence in questing ticks depend in a multifaceted  
88 way on the presence of multiple hosts in suitable tick-habitat throughout the urban ecosystem  
89 (Mackenstedt et al., 2015; Rizzoli et al., 2014). While pathogen transmission and facilitation  
90 are constrained by physiological barriers in hosts and tick vectors, the mobility and presence of  
91 these hosts is shaped by the availability and spatial structuring of habitats. Urban environments  
92 are characterized by severe habitat fragmentation where movement of wildlife can be strongly  
93 constrained – but sometimes also facilitated – by human infrastructures such as buildings, roads

94 and canals. While a number of studies have documented the presence of ticks and tick-borne  
95 pathogens in urban and suburban areas in multiple European cities (Rizzoli et al., 2014), these  
96 have at most compared prevalences among broad urban categories (such as city parks and  
97 suburban forest) (e.g. Hansford et al., 2017; Mehlhorn et al., 2016; Nelson et al., 2015; Rosa et  
98 al., 2018). Other studies have addressed the spatial complexity of ticks and tick-borne pathogen  
99 dynamics at landscape scale in large semi-natural areas (Estrada-Pena, 2003; Kilpatrick et al.,  
100 2017) but we are not aware of any studies that have explicitly linked the presence and  
101 abundance of ticks and tick-borne pathogens to habitat connectivity in an urbanization context.  
102 In this study, we present data on ticks and their pathogens inside green areas along the  
103 urbanization gradient of the metropolitan area of Antwerp (Belgium, Europe) and test whether  
104 this variation can be explained by robust landscape parameters related to urbanization and  
105 habitat connectivity, based on graph theory principles.

106

## 107 **2. Materials and Methods**

### 108 *2.1. Sampling of ticks over the urban-to-rural gradient*

109 Questing ticks were sampled in 22 green spaces ('sampling plots') along an urbanization  
110 gradient in and around Antwerp (Table 1), ranging from the city center to larger forests in the  
111 urban periphery capable of holding viable populations of relevant vertebrate hosts for ticks and  
112 their pathogens (Fig. 1a). For ease of interpretation, areas are designated as urban, suburban or  
113 rural based on their position in or near the urban area (Table 1) but these categories were not  
114 used in the data analysis. Most areas are small to larger public parks combining open spaces  
115 with wooded areas, while some of the more peripheral areas are closed forest. Five green spaces  
116 were included within the central urban area which is entirely separated from the periphery by a  
117 six- to eight-lane semi-circular highway. Ticks were collected by standardized flagging of 10-

118 m transects through leaf litter and low vegetation, representative for the area. Sampling was  
119 done in spring (in two years) and autumn, the two major questing seasons for *I. ricinus* (Gray,  
120 1991). Six plots were sampled in the spring of 2014, 15 were added in the spring of 2016, and  
121 one was added in the autumn of 2016 (Table 1). All questing ticks were collected in Eppendorf  
122 tubes with ethanol (80%) and stored at -20°C until screening. Tick species and age (larva,  
123 nymph, adult) were identified morphologically using stereo-microscope and identification keys  
124 (Heylen et al., 2014; Hillyard, 1996; Manila, 1998). The majority of ticks belonged to *I. ricinus*.  
125 The few individuals that belonged to additional species (*I. frontalis* and *I. hexagonus*) are not  
126 further considered in the analyses.

127 Tick counts were converted to densities, i.e. the number of ticks per square meter for larvae  
128 ('Density Of Larvae': DO-L), nymphs (DO-N) and adults (DO-A).

129

## 130 ***2.2.Pathogen screening***

131 The main focus in this study is on *Borrelia burgdorferi* s.l. (from here onwards '*Borrelia*'),  
132 known to have high infection prevalences in ticks in this part of Belgium. Screening was  
133 focused on the nymphal and adult stage. As vertical *Borrelia* transmission (female tick to  
134 larvae) seldom occurs, unfed larvae are rarely infected (Richter et al., 2012). Nymphal stages  
135 pose the main infection risk to humans (Kilpatrick et al., 2017), while adults often infest  
136 domestic animals (e.g. cats and dogs). All pathogens (including the rarer *B. miyamotoi*,  
137 *Anaplasma phagocytophilum*, *Babesia* spp. and '*C. Neoehrlichia mikurensis*') were screened  
138 by several multiplex real-time qPCR assays (see Heylen et al., 2016 for screening protocols).  
139 *Borrelia* genospecies were further identified by conventional PCR assay targeting the 5S-23S  
140 intergenic region followed by sequencing (Heylen et al., 2013). Based on this data, the density  
141 of *Borrelia* infected ticks could be defined for nymphs ('Density of Infected Nymphs': DI-N)

142 and adults (DI-A), as well as the proportion of *Borrelia* infected nymphs ('Nymphal Infection  
143 Prevalence': N-IP) and adults (A-IP).

144

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### 147 **2.3. *Borrelia* genospecies interpretation**

148 Since unfed larvae are *Borrelia*-free, an infected questing nymph should have obtained the  
149 bacteria from the host on which it fed as larva. While European birds act as reservoirs for the  
150 avian *Borrelia* genospecies (*B. garinii*, *B. valaisiana* and *B. turdi*), mammals carry members of  
151 a different genospecies community (*B. afzelii* and *B. burgdorferi* s.s. and *B. spielmanii*). Thus  
152 the genospecies identified in the infected nymph informs us on which infectious host type the  
153 previous larval stage has fed. Adult ticks can be infected with both mammalian and avian  
154 genospecies, as they have already fed twice (as larva and nymph), hence infections obtained  
155 from adults give us less reliable information on infections in local host populations.

156

### 157 **2.4. Landcover and connectivity measures**

158 Local environmental variables were based on two landcover data layers from the Flemish  
159 Agency for Geographical Information ([www.geopunt.be](http://www.geopunt.be)). Vegetation data were obtained  
160 from the "Groenkaart". Hard surfaces and water bodies were obtained from the GRBgis map.  
161 Landcover data were analysed within a radius of 250 m from the center of each sampling plot  
162 using ArcMap 10.3 (ESRI Inc. 2015). Urbanization was defined as the proportion built-up and  
163 paved area. In addition, we quantified the proportion of water bodies, vegetation above 3 m,  
164 and vegetation below 3 m.



165

166 Landscape connectivity was calculated using Cost Distance (CD) in ArcMap (Spatial Analyst  
167 ArcToolbox), based on the general concept of landscape resistance or ‘friction’. The latter  
168 represents the likelihood that an animal will travel a particular distance through a certain habitat  
169 (Adriaensen et al., 2003). The cost distance between a given location and a predefined source  
170 area is estimated as the minimum cumulative cost associated with the least-cost path between  
171 the two areas, i.e. the path with the lowest overall cost (Fig. 1b). The resistance layer was based  
172 on a landcover map at a 1x1m cell resolution. The resistance values were based on expert  
173 judgement on the effect of landcover on the mobility of a generalized mid-sized terrestrial  
174 vertebrate, with roe deer (*Capreolus capreolus*) as the main target species. Roe deer are  
175 considered as umbrella species for the larger tick hosts on which *I. ricinus* adults feed and  
176 copulate, and have a large impact on *I. ricinus* population in Europe’s low countries (Kilpatrick  
177 et al., 2017). The lowest resistance (value 1) was given to cells covered with tall vegetation (>  
178 3m), followed by low vegetation (value 5) and roads and open water (value 100). Buildings  
179 were absolute barriers (value 0).

180

181 Two cost measures were calculated using different source areas. For the first (CD1), all suitable  
182 patches with vegetation over 3 m high (trees) and minimum 10 ha in size were used as sources,  
183 assuming they contain tick hosts. For the second (CD2), the nearest known roe deer population  
184 was used as a source. To identify these populations, we collected all roe deer observations over  
185 the last 10 years ([www.waarnemingen.be](http://www.waarnemingen.be), data provided by Natuurpunt) and joined  
186 observations with less than 500 m between them. Clusters with less than five observations were  
187 discarded as they possibly represented transient individuals. Cost distances were calculated  
188 from each sampling site to the nearest roe deer observation site that belonged to a cluster (Fig.  
189 1b). Thus, CD1 represents the inverse of connectivity to areas suitable for any host population,

190 while CD2 represents connectivity to known populations of the main tick host. Since CD1 and  
191 CD2 were highly correlated (Pearson's rho: 0.93, N = 22), the first axis of a principal  
192 component analysis was used in further analyses (labelled "cost distance"), explaining 96.7 %  
193 (factor loading CD1: 0.71; f.l. CD2: 0.71).

194

195

196

## 197 ***2.5. Statistical analysis***

198

199 Tick densities (DO-L,-N, -A and DI-N, -A; see above) and the proportion of *Borrelia* infected  
200 ticks (N- and A-IP) were modelled as a function of the following continuous explanatory  
201 variables: urbanization, open water, cultivated area (all in %), plot surface and cost distance.  
202 Generalized estimation equation models (GEE) with exchangeable working correlation were  
203 fitted to the data (see Molenberghs and Verbeke, 2005). These take into account the statistical  
204 dependence of counts in the plots that were repeatedly sampled over several years and/or  
205 seasons. The residuals for count data and proportions were assumed to follow a negative  
206 binomial distribution (log-link) and binomial distribution (logit-link), respectively. Log surface  
207 area at the level of sampling plots was used as offset for the count data.

208 In all models we took into account seasonal and annual variation as fixed effects (categorical  
209 variables). To avoid ill-conditioned information matrices, for each of the models we assessed  
210 the collinearity among explanatory variables following the instructions of "Usage Note  
211 32471"(SAS/Samples & Notes: <http://support.sas.com/kb/32/471.html>) that is based on  
212 (Lesaffre and Marx, 1993; Segerstedt and Nyquist, 1992). Furthermore, we standardized each  
213 of the continuous explanatory variables.

214 The following model restrictions were imposed, because of the limited amount of data  
215 (maximum use of twenty-two independent clusters, i.e. sampling plots) as well as the high  
216 number of tests: (1) No interaction terms among the main explanatory variables were fitted.  
217 Adding them would lead to (almost) saturated models and reduction in statistical power for  
218 each of the tests. (2) Only those variables that were highly significant ( $P < 0.01$ ) are considered  
219 as main results in the discussion section and abstract. A variable that explained part of the  
220 variation, though in a less significant way ( $P < 0.05$ ), was left in the models to remove its  
221 confounder effect. (3) A stepwise backward selection procedure was used to select the best  
222 model. At each step we excluded the fixed factor with the highest non-significant P-value ( $P >$   
223  $0.05$ ), re-ran the model and examined the P-values of the fixed factors in the reduced model.  
224 Model reduction continued until only significant factors ( $P < 0.05$ ) (Steyerberg, 2009). All  
225 estimates are reported as mean  $\pm$  S.E. All data management and statistical analyses were done  
226 in SAS v 9.3 (SAS Institute, Cary, North Carolina, USA).

227

## 228 **3. Results**

### 229 ***3.1. Densities of ticks (DO-L, -N and -A)***

230 An overview of the number of trapped ticks and sampling effort per plot is presented in Table  
231 1. *Ixodes ricinus* was by far the most abundant tick species, but its density strongly varied  
232 among plots (range 0 - 3.8 ticks/m<sup>2</sup>). In the highly urbanized areas within the ring road no ticks  
233 were collected, despite intensive sampling. Tick densities were higher in 2016 than 2014, and  
234 higher in Spring than in Autumn (Table 2). Variation in tick density among sampling plots was  
235 consistent over time, as indicated by correlations for annual counts (Spring 2014 vs. Spring  
236 2016: Spearman correlation = 0.90 for larvae, 0.94 for nymphs, 0.86 for adults; all  $P < 0.02$ ,  $N$   
237 = 6 plots) and seasonal counts (Spring vs. Autumn 2016: 0.81 for larvae, 0.79 for nymphs, 0.67  
238 for adults,  $N = 21$  plots; all  $P$ 's  $< 0.001$ ). Spring and Autumn densities were significantly

239 correlated for all developmental stages (adult vs. larva: 0.49-0.61; larva vs. nymph: 0.65-0.68;  
240 nymph vs. adult: 0.88-0.92; all  $P < 0.022$ ).

241

## 242 **TABLE 1**

243

244 Tick densities were significantly negatively correlated with cost distance (i.e. the inverse of  
245 connectivity) as evidenced by GEE models (Table 2 and Fig. 2, all  $P < 0.035$ ). Furthermore, for  
246 all stages, density was negatively correlated with the level of urbanization (all  $P < 0.003$ ). While  
247 agricultural land cover was shown to be negatively correlated with larval density ( $-1.47 \pm 0.22$   
248 Log counts/m<sup>2</sup>, Z-value: -6.63,  $P = 0.0003$ ), it was positively associated with adult densities  
249 ( $0.39 \pm 0.16$ ; Z-value: 2.49,  $P = 0.013$ ).

250

## 251 **TABLE 2**

252

## 253 **FIGURE 2**

254

### 255 ***3.2.Densities of infected ticks (DI-N, DI-A)***

256 Overviews of the detected pathogens and their prevalences are presented in Table 3. *Borrelia*  
257 was the most prevalent pathogenic agent (overall prevalence nymphs:  $289/1627 = 17.8\%$ ,  
258 adults:  $71/218 = 32.6\%$ ). In 87 % of plots where one or more ticks were trapped, at least one  
259 *Borrelia*-infected tick was found.

260 Factors explaining variation in densities of *Borrelia* infected nymphs and adults were very  
261 similar in sign and effect size to those for overall density of nymphs and adults (Table 2 and  
262 Fig. 2). Overall, the density of *Borrelia*-infected ticks (DI-N and DI-A) was higher in 2016 than

263 in 2014, and higher in Spring than in Autumn. Connectivity and urbanization also had similar  
264 effects on infected ticks compared to overall tick densities.

265

### 266 **TABLE 3**

267

268 When looking into the known host associations of the *Borrelia* genospecies (Table 4), most  
269 ticks were infected with mammalian genospecies (*B. afzelii*, *B. spielmanii* and *B. burgdorferi*  
270 s.s.: 193 out of 267 successfully sequenced individuals = 72%) compared to bird genospecies  
271 (*B. garinii*, *B. valaisiana*; 74 individuals). Unfortunately, the low numbers of successfully  
272 sequenced samples were too low for further analysis on pathogen communities.

273

### 274 **TABLE 4**

275

#### 276 ***3.3. Borrelia infection prevalence (N-IP and A-IP)***

277 For those sampling plots in which at least one tick was collected, the N-IP for 2016 was on  
278 average lower than for 2014, and lower in Autumn than in Spring (Table 3). Factors explaining  
279 variation in infection prevalence of nymphs were dissimilar in sign and effect size to those of  
280 adults (Fig. 3). In nymphs, larger plots ( $0.20 \pm 0.03$ , Z-value = 6.01) with lower agricultural  
281 land cover ( $-0.30 \pm 0.07$ , Z-value = -4.30) showed higher infection prevalence (all  $P < 0.001$ ).  
282 In adults we found the reverse associations (surface area:  $-0.15 \pm 0.07$ , agriculture:  $0.70 \pm 0.17$ ).  
283 Associations with surface area were driven by one important sampling plot in the rural zone  
284 ('Boshhoek'). In addition, open water cover was positively associated with the *Borrelia*  
285 prevalence ( $0.37 \pm 0.10$ , Z-value = 3.63,  $P < 0.001$ ).

286 Overall prevalences of the rarer pathogenic agents (*A. phagocytophilum* , ‘*Ca N. mikurensis*’,  
287 *Babesia* sp. and *B. miyamotoi*) were all  $\leq 5$  % in nymphs and  $\leq 10$  % in adults, with no marked  
288 variation among those plots where more than 10 ticks were screened (Appendix 1). 38 ticks (28  
289 nymphs, 10 adults) were co-infected (i.e. carried more than one pathogen), with *Borrelia* x ‘*Ca*  
290 *N. mikurensis*’ (N = 12), *Borrelia* x *A. phagocytophilum* (N = 11) and *Borrelia* x *B. miyamotoi*  
291 (N = 10) as the three most common combinations. Three tick individuals were infected with  
292 three pathogenic agents each.

293

294

295 **FIGURE 3**

296

#### 297 **4. Discussion**

298 While many studies have previously reported the presence of ticks and tick-borne pathogens in  
299 urban or peri-urban areas (Hansford et al., 2017; Nelson et al., 2015; Oechslin et al., 2017;  
300 Rizzoli et al., 2014), we are not aware of previous studies that have analyzed the density of  
301 questing ticks in an explicit spatial context, taking into account not only properties of green  
302 spaces but also the surrounding landcover and the proximity to other green spaces. We found  
303 that tick densities decreased with urbanization and increased with the degree of connectivity to  
304 potential source host populations. Densities of ticks infected with *Borrelia burgdorferi* s.l.  
305 followed the same pattern. The variation in *Borrelia* prevalence in those plots where ticks were  
306 found, was partly explained by factors other than connectivity and urbanization (plot size,  
307 agricultural cover, and water cover) and the signs and effect sizes of these variables differed  
308 between nymphs and adults.

309

#### 310 ***4.1.Density of (infected) ticks***

311 Despite the large number of studies documenting the presence of ticks in urban and suburban  
312 areas, most of these have not quantified variation in tick abundance (e.g. studies collecting a  
313 fixed amount of ticks for pathogen detection, such as Tappe et al., 2014) and/or focus only on  
314 sites with ticks known to be present (e.g. Junttila et al., 1999) or were based on small samples  
315 per location (e.g. Hansford et al., 2017) or a limited number of sites (e.g. Cekanac et al., 2010;  
316 Kazimirova et al., 2016; Nelson et al., 2015). Other studies have reported on variation in tick  
317 abundance among different habitat types, but without taking into account the surrounding  
318 landscape (e.g. Hornok et al., 2014; Krstic et al., 2016; Maetzel et al., 2005; Rosa et al., 2018).  
319 Some of these studies suggest similar trends as in our study with lower abundance or presence  
320 of ticks in urban parks or other green spaces close to urban centers (e.g. Cekanac et al., 2010;  
321 Hansford et al., 2017; Maetzel et al., 2005; Oechslin et al., 2017; Pangracova et al., 2013), but  
322 without further quantitative details or analysis on the driving landscape characteristics. In a  
323 study including urban, agricultural and natural areas in five countries, Rosa et al. (2018)  
324 concluded that there was no significant difference among these three habitats when accounting  
325 for variation in Normalized Difference Vegetation Index (NDVI) and precipitation; however,  
326 it should be noted that several of the included urban parks were of large size and/or associated  
327 with small urban centers compared to our study.

328 The significant effects of urbanization and connectivity on *I. ricinus* density in our study can  
329 be readily explained by this tick's biology. The presence of larger mammals (roe deer, foxes,  
330 wild boars and others) on which adult developmental stages feed and copulate, is one of the  
331 main conditions for viable *I. ricinus* populations (Kilpatrick et al., 2017; Rizzoli et al., 2014).  
332 As one engorged fertilized adult female will give rise to more than 2000 larvae (Balashov, 1972;  
333 Gray, 1991; Gray, 1998), even a brief stop-over of an infested end host can initiate or boost a  
334 local *I. ricinus* population. Urban areas, and in particular city centers, typically have reduced

335 diversity of wildlife (Aronson et al., 2016; Nielsen et al., 2014) although densities of so-called  
336 urban adapters may be as high or even higher than in rural areas (Mackenstedt et al., 2015;  
337 Rizzoli et al., 2014). At least part of the reason for the loss of species is the fragmented habitat  
338 and high resistance of the urban matrix for wildlife movement, leading to strong isolation of  
339 these areas from each other and from the putative source areas in rural areas outside the city  
340 (e.g. Braaker et al., 2017; Nielsen et al., 2014; Verbeylen et al., 2003). Thus, areas characterized  
341 by low connectivity (high cost distance value) such as the highly urbanized areas within the  
342 Antwerp ring road, may still hold populations of smaller and/or more mobile hosts such as  
343 rodents or birds, but not of larger animals such as roe deer that are major hosts of adults.

344 Our initial aim in this study was to discriminate between the importance of local connectivity  
345 (i.e. to nearby green spaces) and connectivity to major forested areas with known important  
346 host populations, i.e. roe deer. The first measure would then reflect the importance of intra-  
347 urban host populations (typically small and medium-sized mammals and birds) and/or the  
348 importance of such urban green spaces as stepping stones for larger dispersing hosts, such as  
349 occasional movements of roe deer or foxes into the (sub-)urban area. However, since the two  
350 connectivity measures proved to be more strongly correlated than we anticipated, we were  
351 unable to make this distinction. The strong correlation indicates that green spaces close to the  
352 urban center are not only far from peripheral source areas, but also mutually strongly isolated.  
353 In contrast, suburban green spaces are closer to the periphery but also appear to have more  
354 interconnections between them. This pattern clearly reflects the lack of extensive green  
355 corridors into the city center. Other metropolitan areas with a different layout of green spaces  
356 may provide better opportunities to disentangle the effects of local versus long-distance  
357 connectivity.

358 The negative association between tick density and urbanization can further be explained by  
359 abiotic contrasts associated with the urban-rural gradient. Because immature developmental



360 stages - larvae in particular - are very vulnerable to desiccation (Kahl and Knülle, 1988;  
361 Kilpatrick et al., 2017; Perret et al., 2000) they live close to the ground where humidity is high.  
362 Rural green spaces with trees and bushes are less intensively managed and contain more  
363 extensive litter layers that protect ticks against dry weather conditions. Urbanized areas are drier  
364 and less favorable for tick survival, due to the radiation of surrounding concrete infrastructure,  
365 paved and graveled surfaces that do not absorb water nor gradually release humidified air.  
366 Because of the drier air (hence lower heat), ticks experience stronger temperature variations  
367 that are detrimental to their survival (Herrmann and Gern, 2013). In addition, we found that the  
368 density of larval ticks (but not nymphs or adults) decreased with agricultural land cover. This  
369 could indicate that larval survival is particularly susceptible to the microclimatological  
370 conditions associated with farmlands (e.g. longer periods of drought and sun exposure)  
371 (Herrmann and Gern, 2010). On the other hand, low larval densities compared to nymphs or  
372 adults may reflect the lower abundance of propagation hosts such as roe deer relative to small  
373 and mid-sized hosts on which larvae and nymphs may feed. Indeed, in many of the suburban  
374 areas we found few larvae compared to nymphs or even adults. This may to some extent reflect  
375 sampling variation, as clusters of larvae may be overlooked when sampling, especially if  
376 densities are low, while nymphs and adults can be expected to be more randomly distributed  
377 after dropping off from their feeding hosts. Nevertheless, overall the ratio of nymphs to larvae  
378 and adults to larvae were much higher in the suburban areas ( $A/L = 0.05$ ;  $N/L = 0.21$ ) than in  
379 the rural areas ( $A/L: 0.015$ ;  $N/L = 0.14$ ). High adult to nymph ratios were also reported in  
380 suburban areas in Eastern Slovakia (Pangracova et al., 2013).

381

#### 382 **4.2. *Borrelia burgdorferi* s.l. prevalence in tick infested areas**

383 In areas where ticks were present, the *Borrelia* infection rates did not show any association with  
384 degree of urbanization. This is in line with previous studies showing infection rates in urban

385 areas to be comparable to those outside the city (cf. Rizzoli et al., 2014). Larger plots tended to  
386 have significantly higher infection prevalence, but this association was driven by a single rural  
387 area ('Boshoek'). Associations with prevalence (including agricultural land and water cover)  
388 are driven by the spatial differences in exposure levels, susceptibility, or infectiousness within  
389 and between host and vector species populations. In addition, local tick and host birth and  
390 mortality rates can be affected by the pathogen itself, resulting in prevalence differences.  
391 Currently we lack knowledge on local host communities, their population dynamics and host  
392 species interactions with *Borrelia*, making the explanation of the observed patterns very  
393 speculative.

394 Similar proportions of avian and mammalian *Borrelia* genospecies were found in suburban and  
395 rural areas. Even though sample sizes are too limited to warrant further analyses, the data do  
396 not support the idea that ticks in the most urban areas depend more strongly on avian versus  
397 mammalian hosts. Furthermore, the comparatively high prevalence of mammalian genospecies  
398 such as *Borrelia afzelii* suggests that even small suburban green spaces are capable of  
399 supporting enzootic cycles in local mammal populations, despite the overall low tick  
400 abundance. We cannot rule out the possibility that such cycles are supported at least in part by  
401 other tick species, notably the burrow-dwelling *Ixodes hexagonus* living on hedgehogs and  
402 competent for transmitting mammalian *Borrelia* genospecies as well (Jahfari et al., 2017).  
403 Besides *Borrelia*, we traced several rarer pathogenic agents in questing ticks. Although poorly  
404 studied, exposure to and possibly also infection with multiple tick-borne pathogens in humans  
405 in this area seems to be the rule rather than the exception (Heylen et al., 2017).

406

#### 407 ***4.3. Concluding remarks***

408 We showed that landscape connectivity and urbanization significantly influence the variation  
409 in tick abundances (DO-L, -N, -A) and hence the *Borrelia*-exposure to humans (DI-N and -A)

410 along an urbanization gradient. Tick densities are strongly reduced with increasing urbanization  
411 and distance from source areas. However, *Borrelia* prevalences are comparable along the urban-  
412 rural gradient, and even moderately isolated suburban green spaces may support enzootic cycles  
413 of different *Borrelia* genospecies. Our results suggest that the risk of tick-borne infections  
414 should be taken into account when designing green spaces in urban areas. In particular,  
415 improving connectivity for large propagation hosts such as roe deer, and/or creating additional  
416 habitat for these species in proximity to urban areas, may lead to a higher risk in urban areas.  
417 Our study also highlights that urban and suburban areas should be included in surveillance for  
418 tick-borne diseases and public health campaigns (Uspensky, 2014) because, even though tick  
419 densities may be low, tick infection prevalence and the risk of human contact are high. Still,  
420 substantial variation in tick density remains unexplained. Future research needs to include host  
421 surveys as well as more detailed habitat characteristics and specific resources (e.g. bird feeders,  
422 waste) that affect host population densities. Understanding the mechanisms how spatial factors  
423 affect pathogen risk in urban areas also requires more detailed knowledge on how wildlife  
424 presence and movements respond to green space characteristics such as connectivity, habitat  
425 quality and human disturbance.

426

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## 441 **References**

442

443 Adriaansen F, Chardon JP, De Blust G, Swinnen E, Villalba S, Gulinck H, et al. The application of  
444 'least-cost' modelling as a functional landscape model. *Landscape and Urban Planning* 2003;  
445 64: 233-247.

446 Aronson MFJ, Nilon CH, Lepczyk CA, Parker TS, Warren PS, Cilliers SS, et al. Hierarchical filters  
447 determine community assembly of urban species pools. *Ecology* 2016; 97: 2952-2963.

448 Balashov YS. Bloodsucking ticks (Ixodidea) - vectors of diseases of man and animals. *Miscellaneous*  
449 *Publications of the Entomological Society of America* 1972; 8: 159-376.

450 Braaker S, Kormann U, Bontadina F, Obrist MK. Prediction of genetic connectivity in urban  
451 ecosystems by combining detailed movement data, genetic data and multi-path modelling.  
452 *Landscape and Urban Planning* 2017; 160: 107-114.

453 Braks MAH, Van Wieren SE, Takken W, Sprong H. *Ecology and prevention of Lyme borreliosis:*  
454 *Wageningen Academic Publishers, 2016.*

455 Cekanac R, Pavlovic N, Gledovic Z, Grgurevic A, Stajkovic N, Lepsanovic Z, et al. Prevalence of  
456 *Borrelia burgdorferi* in *Ixodes ricinus* Ticks in Belgrade Area. Vector-Borne and Zoonotic  
457 Diseases 2010; 10: 447-452.

458 Estrada-Pena A. The relationships between habitat topology, critical scales of connectivity and tick  
459 abundance *Ixodes ricinus* in a heterogeneous landscape in northern Spain. *Ecography* 2003;  
460 26: 661-671.

461 Gern L, Rais O. Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks  
462 (Acari: Ixodidae). *Journal of Medical Entomology* 1996; 33: 189-192.

463 Gray JS. The development and seasonal activity of the tick *Ixodes ricinus*: a vector of Lyme  
464 borreliosis. *Review of Medical and Veterinary Entomology* 1991; 79: 323-333.

465 Gray JS. The ecology of ticks transmitting Lyme borreliosis. *Experimental & Applied Acarology*  
466 1998; 22: 249-258.

467 Haase D, Larondelle N, Andersson E, Artmann M, Borgstrom S, Breuste J, et al. A Quantitative  
468 Review of Urban Ecosystem Service Assessments: Concepts, Models, and Implementation.  
469 *Ambio* 2014; 43: 413-433.

470 Hansen R, Pauleit S. From Multifunctionality to Multiple Ecosystem Services? A Conceptual  
471 Framework for Multifunctionality in Green Infrastructure Planning for Urban Areas. *Ambio*  
472 2014; 43: 516-529.

473 Hansford KM, Fonville M, Gillingham EL, Coipan EC, Pietzsch ME, Krawczyk AI, et al. Ticks and  
474 *Borrelia* in urban and peri-urban green space habitats in a city in southern England. *Ticks and*  
475 *Tick-Borne Diseases* 2017; 8: 353-361.

476 Herrmann C, Gern L. Survival of *Ixodes ricinus* (Acari: Ixodidae) under challenging conditions of  
477 temperature and humidity is influenced by *Borrelia burgdorferi* sensu lato Infection. *Journal*  
478 *of Medical Entomology* 2010; 47: 1196-1204.

479 Herrmann C, Gern L. Survival of *Ixodes ricinus* (Acari: Ixodidae) nymphs under cold conditions is  
480 negatively influenced by frequent temperature variations. *Ticks and tick-borne disease* 2013;  
481 4: 445-451.

482 Heylen D, De Coninck E, Jansen F, Madder M. Differential diagnosis of three common Ixodes spp.  
483 ticks infesting songbirds of Western Europe: *Ixodes arboricola*, *I. frontalis* and *I. ricinus*.  
484 Ticks and Tick-Borne Diseases 2014; 5: 693-700.

485 Heylen D, Fonville M, Docters van Leeuwen A, Stroo A, Duisterwinkel M, van Wieren S, et al.  
486 Pathogen communities of songbird-derived ticks in Europe's low countries. Parasites &  
487 Vectors 2017; 10.

488 Heylen D, Fonville M, van Leeuwen AD, Sprong H. Co-infections and transmission dynamics in a  
489 tick-borne bacterium community exposed to songbirds. Environmental Microbiology 2016;  
490 18: 988-996.

491 Heylen D, Tijssse E, Fonville M, Matthysen E, Sprong H. Transmission dynamics of *Borrelia*  
492 *burgdorferi* s.l. in a bird tick community. Environ.Microbiol. 2013; 15: 663-673.

493 Hillyard PD. Ticks of North-West Europe. London: Backhuys Publishers, 1996.

494 Hornok S, Meli ML, Gonczi E, Halasz E, Takacs N, Farkas R, et al. Occurrence of ticks and  
495 prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* s.l. in three types of  
496 urban biotopes: Forests, parks and cemeteries. Ticks and Tick-Borne Diseases 2014; 5: 785-  
497 789.

498 Jahfari S, Ruyts SC, Frazer-Mendelewska E, Jaarsma R, Verheyen K, Sprong H. Melting pot of tick-  
499 borne zoonoses: the European hedgehog contributes to the maintenance of various tick-borne  
500 diseases in natural cycles urban and suburban areas. Parasites & Vectors 2017; 10.

501 Junttila T, Peltomaa M, Soini H, Marjamaki M, Viljanen MK. Prevalence of *Borrelia burgdorferi* in  
502 *Ixodes ricinus* ticks in urban recreational areas of Helsinki. Journal of Clinical Microbiology  
503 1999; 37: 1361-1365.

504 Kahl O, Knülle W. Water vapour uptake from subsaturated atmosphere by engorged immature ixodid  
505 ticks. Experimental and applied acarology 1988; 4.

506 Kazimirova M, Hamsikova Z, Kocianova E, Marini G, Mojsova M, Mahrikova L, et al. Relative  
507 density of host-seeking ticks in different habitat types of south-western Slovakia.  
508 Experimental and Applied Acarology 2016; 69: 205-224.

509 Kilpatrick AM, Dobson ADM, Levi T, Salkeld DJ, Swei A, Ginsberg HS, et al. Lyme disease ecology  
510 in a changing world: consensus, uncertainty and critical gaps for improving control.  
511 Philosophical Transactions of the Royal Society B-Biological Sciences 2017; 372: 15.

512 Krstic M, Stajkovic N, Lazic S. Prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks  
513 and assessment of entomological risk index at localities in Belgrade. *Vojnosanitetski Pregled*  
514 2016; 73: 817-824.

515 Kurtenbach K, Sewell HS, Ogden NH, Randolph SE, Nuttall PA. Serum complement sensitivity as a  
516 key factor in Lyme disease ecology. *Infection and Immunity* 1998; 66: 1248-1251.

517 Lesaffre E, Marx BD. Collinearity in Generalized Linear Regression. *Communications in Statistics -*  
518 *Theory and Methods* 1993; 22: 1933-1952.

519 LoGiudice K, Ostfeld RS, Schmidt K, Keesing F. The ecology of infectious disease: effects of host  
520 diversity and community composition on Lyme disease risk. *Proc. Natl. Acad. Sci. U. S. A.*  
521 2003; 100: 567–571.

522 Mackenstedt U, Jenkins D, Romig T. The role of wildlife in the transmission of parasitic zoonoses in  
523 peri-urban and urban areas. *International Journal for Parasitology-Parasites and Wildlife* 2015;  
524 4: 71-79.

525 Maetzel D, Maier WA, Kampen H. *Borrelia burgdorferi* infection prevalences in questing *Ixodes*  
526 *ricinus* ticks (Acari : Ixodidae) in urban and suburban Bonn, western Germany. *Parasitology*  
527 *Research* 2005; 95: 5-12.

528 Manila G. *Acari, Ixodida*. Bologna, 1998.

529 Matuschka FR, Fisher P, Heiler M, Richter D, Spielman A. Capacity of European animals as reservoir  
530 hosts for the Lyme disease spirochete. . *Journal of Infectious Diseases* 1992; 165: 479–483.

531 Mehlhorn H, Mehlhorn T, Muller M, Vogt M, Rissland J. Tick survey for prevalent pathogens in peri-  
532 urban recreation sites in Saarland and Rhineland-Palatinate (Germany). *Parasitology Research*  
533 2016; 115: 1167-1172.

534 Molenberghs G, Verbeke G. *Models for Discrete Longitudinal Data*. Berlin-Heidelberg-New York  
535 Springer-Verlag, 2005.

536 Nelson C, Banks S, Jeffries CL, Walker T, Logan JG. Tick abundances in South London parks and the  
537 potential risk for Lyme borreliosis to the general public. *Medical and Veterinary Entomology*  
538 2015; 29: 448-452.

539 Nielsen AB, van den Bosch M, Maruthaveeran S, van den Bosch CK. Species richness in urban parks  
540 and its drivers: A review of empirical evidence. *Urban Ecosystems* 2014; 17: 305-327.

541 Oechslin CP, Heutschi D, Lenz N, Tischhauser W, Peter O, Rais O, et al. Prevalence of tick-borne  
542 pathogens in questing *Ixodes ricinus* ticks in urban and suburban areas of Switzerland.  
543 *Parasites & Vectors* 2017; 10: 18.

544 Pangracova L, Derdakova M, Pekarik L, Hviscova I, Vichova B, Stanko M, et al. *Ixodes ricinus*  
545 abundance and its infection with the tick-borne pathogens in urban and suburban areas of  
546 Eastern Slovakia. *Parasites & Vectors* 2013; 6: 8.

547 Perini K, Sabbion P. Ecosystem Services in Urban Areas – Social, Environmental, and Economic  
548 Benefit. In: *Urban Sustainability and River Restoration: Green and Blue Infrastructure*: John  
549 Wiley & Sons, 2016.

550 Perret JL, Guigoz E, Rais O, Gern L. Influence of saturation deficit and temperature on *Ixodes ricinus*  
551 tick questing activity in a Lyme borreliosis-endemic area (Switzerland). *Parasitology Research*  
552 2000; 86: 554-557.

553 Piesman J, Gern L. Lyme borreliosis in Europe and North America. *Parasitology* 2004; 129: S191-  
554 S220.

555 Richter D, Debski A, Hubalek Z, Matuschka FR. Absence of Lyme disease spirochetes in larval  
556 *Ixodes ricinus* ticks. *Vector-Borne and Zoonotic Diseases* 2012; 12: 21-27.

557 Rizzoli A, Silaghi C, Obiegala A, Rudolf I, Hubalek Z, Foldvari G, et al. *Ixodes ricinus* and its  
558 transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for  
559 public health. *Frontiers in Public Health* 2014; 2: 251-277.

560 Rosa R, Andreo V, Tagliapietra V, Barakova I, Arnoldi D, Hauffe HC, et al. Effect of Climate and  
561 Land Use on the Spatio-Temporal Variability of Tick-Borne Bacteria in Europe. *International*  
562 *Journal of Environmental Research and Public Health* 2018; 15: 15.



- 563 Segerstedt B, Nyquist H. On the conditioning problem in generalized linear models. Journal of  
564 Applied Statistics 1992; 19: 513-526.
- 565 Steyerberg EW. Clinical Prediction Models:: A Practical Approach to Development, Validation, and  
566 Updating. New York: Springer, 2009.
- 567 Tappe J, Jordan D, Janecek E, Fingerle V, Strube C. Revisited: *Borrelia burgdorferi* sensu lato  
568 infections in hard ticks (*Ixodes ricinus*) in the city of Hanover (Germany). Parasites & Vectors  
569 2014; 7: 10.
- 570 Uspensky I. Tick pests and vectors (Acari: Ixodoidea) in European towns: Introduction, persistence  
571 and management. Ticks and Tick-Borne Diseases 2014; 5: 41-47.
- 572 Verbeylen G, De Bruyn L, Adriaensen F, Matthysen E. Does matrix resistance influence Red squirrel  
573 (*Sciurus vulgaris* L. 1758) distribution in an urban landscape? Landscape Ecology 2003; 18:  
574 791-805.
- 575 Voordouw MJ. Co-feeding transmission in Lyme disease pathogens. Parasitology 2015; 142: 290-302.
- 576 Zipperer WC, Pickett STA. Urban Ecology: Patterns of Population Growth and Ecological Effects.:  
577 John Wiley & Sons, 2012.