



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



D. Heylen^{a,b,*}, R. Lasters^c, F. Adriaensen^c, M. Fonville^d, H. Sprong^d, E. Matthysen^c

^a Interuniversity Institute for Biostatistics and statistical Bioinformatics, Hasselt University, Diepenbeek, Belgium

^b Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, United States of America

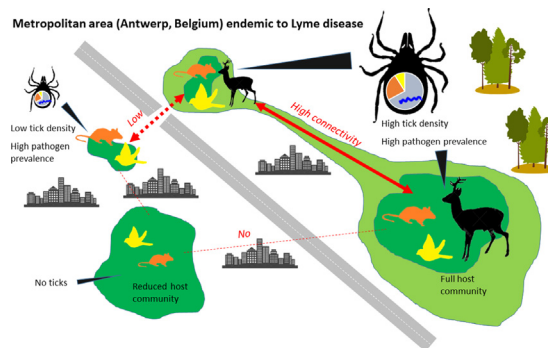
^c Department of Biology, University of Antwerp, Wilrijk, Belgium

^d Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and Environment (RIVM), Bilthoven, the Netherlands

HIGHLIGHTS

- Landscape effects on pathogen flows are poorly understood in urban areas.
- Green space characteristics based on Graph Theory principles were quantified.
- Collected ticks over an urbanization gradient were screened for Lyme disease.
- Connectivity and plot size affected tick density and disease prevalence.
- Tick-borne infections should be taken into account when designing green spaces.

GRAPHICAL ABSTRACT



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ABSTRACT

Green spaces in the city are important for human wellbeing, but are also zones in which humans can become infected with zoonotic diseases. Therefore, there is a need to understand how infection risk is related to green space characteristics, wildlife communities and connectivity with rural areas hosting reservoir populations of hosts. Our hypothesis is that wildlife hosts in urban green spaces, and thereby the prevalence of questing ticks and their Lyme disease causing pathogens (*Borrelia burgdorferi* s.l.), can be partly predicted based on green space characteristics as well as measures of connectivity to known source areas. We sampled ticks in twenty-two green spaces during Spring (2014 and 2016) and Autumn 2016, located along an urbanization gradient in Antwerp (Belgium). More than 18,000 m² was sampled, with tick densities ranging from 0 to 386 individuals/100 m². We estimated connectivity using the least-cost algorithm as either the cost distance to the nearest green space, or to a known population of roe deer (*Capreolus capreolus*), known to be an important tick propagation host. Both connectivity measures turned out to be correlated, reflecting a gradient in green space isolation from the periphery to the urban center. In 87% of plots where ticks were trapped, at least one *Borrelia*-infected tick was found. The overall *Borrelia*-prevalence in nymphs was 17.8%, in adults 32.6%. Density of infected ticks decreased with urbanization and increased with connectivity. Nymphs in larger green spaces were more likely to be infected. While density and infection prevalence for adults increased with the amount of neighboring agricultural land, the larval density and nymphal infection prevalence decreased. Interestingly, the proportion of *Borrelia* genospecies associated with birds or mammals was comparable in rural and (sub)urban areas (bird/mammal: 0.38), suggesting that even in small green spaces *Borrelia* infections can persist in local host populations.

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* Corresponding author at: Princeton University, 212 Eno Hall, NJ 08544 Princeton, U.S.A.

E-mail address: DHeylen@princeton.edu (D. Heylen).

1. Introduction

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

Hard ticks (Ixodidae) are important vectors of human and animal pathogens in the temperate climate zone, notably of Lyme borreliosis, the most prominent among tick-borne diseases (TBD). In Europe, the main tick vector is *Ixodes ricinus* (Gray, 1998), which is increasingly observed in urbanized environments with pathogen prevalences that may be as high as those in rural sites (Rizzoli et al., 2014; Uspensky, 2014). Ixodid ticks feed once in each life stage on a different host individual, usually a different species due to the tick's exophilic biology. Since *I. ricinus* is a generalist parasite, the majority of wild as well as domestic animals present in urban environments can potentially serve as tick-maintenance hosts, and in turn get exposed to a variety of pathogens to which they may be susceptible or (become) resistant. Especially in suburban areas, medium to large sized mammals (e.g. fox, deer, hedgehogs) occur, which are *I. ricinus* end-hosts, and therefore these areas may act as population sources for *I. ricinus* (e.g. Rizzoli et al., 2014). Mobile hosts such as birds may also introduce ticks to locations where they may pose a risk to humans, even if no suitable hosts are available to complete a full lifecycle.

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

Thus, local tick abundances and *Borrelia* prevalence in questing ticks depend in a multifaceted way on the presence of multiple hosts in suitable tick-habitat throughout the urban ecosystem (Mackenstedt et al., 2015; Rizzoli et al., 2014). While pathogen transmission and facilitation are constrained by physiological barriers in hosts and tick vectors, the mobility and presence of these hosts is shaped by the availability and spatial structuring of habitats. Urban environments are characterized by severe habitat fragmentation where movement of wildlife can be strongly constrained – but sometimes also facilitated – by human infrastructures such as buildings, roads and canals. While a number of studies have documented the presence of ticks and tick-borne pathogens in urban and suburban areas in multiple European cities (Rizzoli et al., 2014), these have at most compared prevalences among broad urban categories (such as city parks and suburban forest) (e.g. Hansford et al., 2017; Mehlhorn et al., 2016; Nelson et al., 2015; Rosa et al., 2018). Other studies have addressed the spatial complexity of ticks and tick-borne pathogen dynamics at landscape scale in large semi-

natural areas (Estrada-Pena, 2003; Kilpatrick et al., 2017) but we are not aware of any studies that have explicitly linked the presence and abundance of ticks and tick-borne pathogens to habitat connectivity in an urbanization context. In this study, we present data on ticks and their pathogens inside green areas along the urbanization gradient of the metropolitan area of Antwerp (Belgium, Europe) and test whether this variation can be explained by robust landscape parameters related to urbanization and habitat connectivity, based on graph theory principles.

2. Materials and methods

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

Questing ticks were sampled in 22 green spaces ('sampling plots') along an urbanization gradient in and around Antwerp (Table 1), ranging from the city center to larger forests in the urban periphery capable of holding viable populations of relevant vertebrate hosts for ticks and their pathogens (Fig. 1a). For ease of interpretation, areas are designated as urban, suburban or rural based on their position in or near the urban area (Table 1) but these categories were not used in the data analysis. Most areas are small to larger public parks combining open spaces with wooded areas, while some of the more peripheral areas are closed forest. Five green spaces were included within the central urban area which is entirely separated from the periphery by a six- to eight-lane semi-circular highway. Ticks were collected by standardized flagging of 10-m transects through leaf litter and low vegetation, representative for the area. Sampling was done in spring (in two years) and autumn, the two major questing seasons for *I. ricinus* (Gray, 1991). Six plots were sampled in the spring of 2014, 15 were added in the spring of 2016, and one was added in the autumn of 2016 (Table 1). All questing ticks were collected in Eppendorf tubes with ethanol (80%) and stored at -20°C until screening. Tick species and age (larva, nymph, adult) were identified morphologically using stereo-microscope and identification keys (Heylen et al., 2014; Hillyard, 1996; Manila, 1998). The majority of ticks belonged to *I. ricinus*. The few individuals that belonged to additional species (*I. frontalis* and *I. hexagonus*) are not further considered in the analyses.

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

2.2. Pathogen screening

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

2.3. *Borrelia* genospecies interpretation

Since unfed larvae are *Borrelia*-free, an infected questing nymph should have obtained the bacteria from the host on which it fed as larva. While European birds act as reservoirs for the avian *Borrelia*

Table 1

Ixodes ricinus tick density and sampling effort in twenty-two plots in the metropolitan area of Antwerp. Areas are arranged by urbanization category and size.

ID	Plot (timing of sampling)	Coordinates	Urb ^a	Size (ha)	Sample effort (m ²)	#/100 m ² (# <i>I. ricinus</i> individuals collected)				Other ixodid ticks ^b
						Larva	Nymph	Adult	Total	
1	Boshhoek (***)	51°07'N, 4°30'E	ru	427	2000	346.15(6923)	35.4(708)	4.55(91)	386.1(7722)	
2	Peerdsbos (**)	51°16'N, 4°29'E	ru	147	1080	227.13(2453)	46.3(500)	3.61(39)	277.04(2992)	IF:1A
3	Oude Spoorwegberm-A (*)	51°06'N, 4°27'E	ru	17.2	40	0(0)	30(12)	15(6)	45(18)	
4	Oude Spoorwegberm-B (**)	51°07'N, 4°27'E	ru	5.8	280	2.86(8)	26.07(73)	1.79(5)	30.71(86)	
5	Park Lint (***)	51°07'N, 4°29'E	ru	3.1	560	0.71(4)	3.39(19)	0.18(1)	4.29(24)	
6	Park Heuvelhof (**)	51°10'N, 4°29'E	ru	2.4	1180	0(0)	1.1(13)	0.17(2)	1.27(15)	IH:2N
7	George v Raemdonckpark (**)	51°09'N, 4°29'E	ru	1.2	500	1(5)	0.6(3)	0.2(1)	1.8(9)	
8	Rivierenhof (**)	51°13'N, 4°28'E	su	132	960	77.4(743)	10.42(100)	4.17(40)	91.98(883)	IF:1N;1A
9	Domein Vogelzang (**)	51°11'N, 4°24'E	su	40	520	9.23(48)	0.19(1)	0(0)	9.42(49)	
10	Beeldenmuseumpark (***)	51°10'N, 4°25'E	su	24	2640	13.14(347)	5.04(133)	0.76(20)	18.94(500)	IF:1A
11	FortVI (**)	51°09'N, 4°24'E	su	17.8	540	5(27)	3.52(19)	0.37(2)	8.89(48)	IF:1N/IH:2N
12	ParkSorghvliedt (***)	51°10'N, 4°21'E	su	16.1	2200	0(0)	1.27(28)	0.23(5)	1.5(33)	
13	Wolvenberg (**)	51°12'N, 4°26'E	su	11.4	680	0(0)	0(0)	0(0)	0(0)	
14	Boekenbergpark (**)	51°12'N, 4°27'E	su	10.3	1680	0.06(1)	0.36(6)	0.12(2)	0.54(9)	
15	Brilschanspark (**)	51°11'N, 4°26'E	su	9.7	360	0.28(1)	0(0)	0(0)	0.28(1)	
16	Mastvest (**)	51°11'N, 4°23'E	su	7.4	140	0(0)	0(0)	0(0)	0(0)	
17	Steytelinckpark (**)	51°10'N, 4°24'E	su	7.1	360	17.78(64)	2.5(9)	0.28(1)	20.56(74)	
18	Leeuwerikpark (**)	51°11'N, 4°25'E	su	3	340	59.12(201)	0.88(3)	0.59(2)	60.59(206)	
19	Park Spoor Noord (**)	51°13'N, 4°25'E	u	17	220	0(0)	0(0)	0(0)	0(0)	
20	Stadspark (***)	51°12'N, 4°25'E	u	11.1	1040	0(0)	0(0)	0(0)	0(0)	
21	Hof van Leysen (**)	51°12'N, 4°24'E	u	6.5	260	0(0)	0(0)	0(0)	0(0)	
22	Koning Albertpark (***)	51°11'N, 4°24'E	u	6.1	480	0(0)	0(0)	0(0)	0(0)	

Timing sampling: ***Spring (2014 and 2016) and Autumn 2016; **Spring and Autumn 2016; *Autumn 2016.

^a Urbanisation level: rural (ru), suburban (su), urban (u).

^b Other ixodid ticks: *Ixodes frontalis* (IF); *Ixodes hexagonus* (IH); Larva (L); Nymph (N); Adult (A).

genospecies (*B. garinii*, *B. valaisiana* and *B. turdi*), mammals carry members of a different genospecies community (*B. afzelii* and *B. burgdorferi* s. s. and *B. spielmanii*). Thus the genospecies identified in the infected nymph informs us on which infectious host type the previous larval stage has fed. Adult ticks can be infected with both mammalian and avian genospecies, as they have already fed twice (as larva and nymph), hence infections obtained from adults give us less reliable information on infections in local host populations.

2.4. Landcover and connectivity measures

Local environmental variables were based on two landcover data layers from the Flemish Agency for Geographical Information (www.geopunt.be). Vegetation data were obtained from the “Groenkaart”. Hard surfaces and water bodies were obtained from the GRBgis map. Landcover data were analyzed within a radius of 250 m from the center of each sampling plot using ArcMap 10.3 (ESRI Inc. 2015). Urbanization

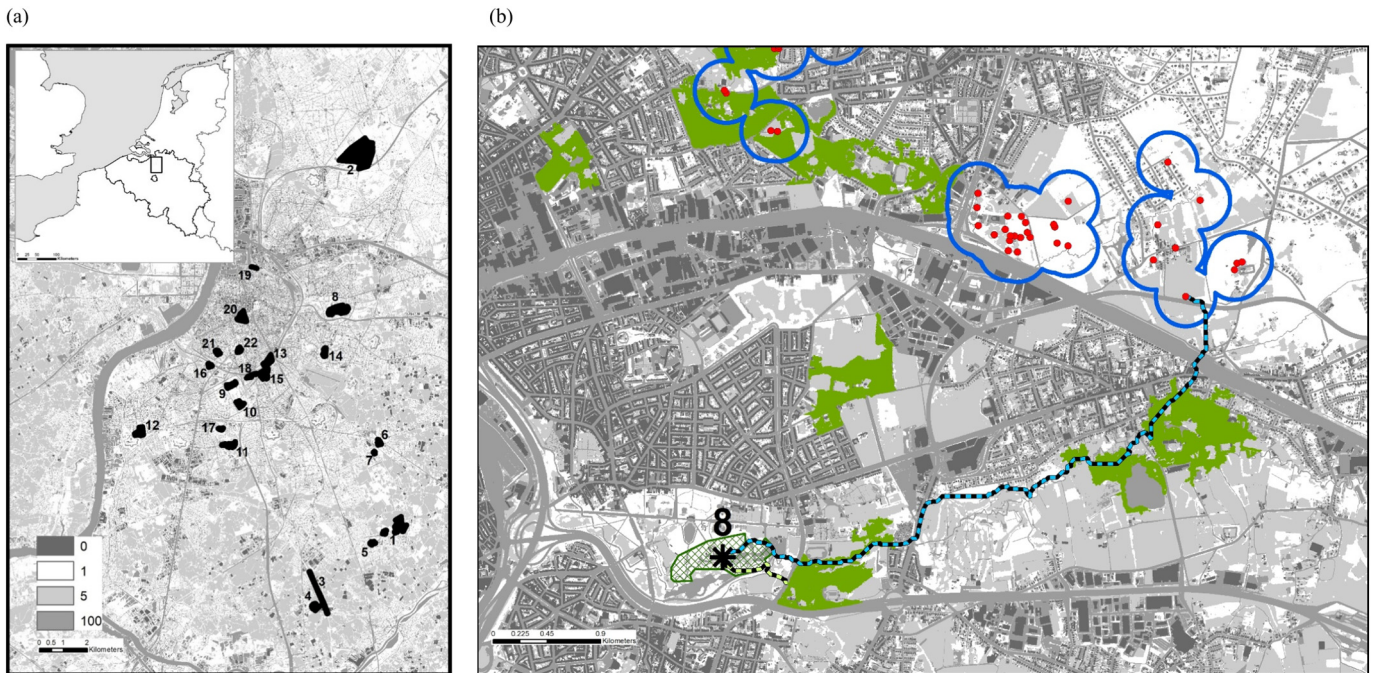


Fig. 1. (a) Study area with sampling sites (numbered as in Table 1). (b) Example of least-cost paths between sampling area 12 and nearest source areas. Grayscales in both (a) and (b) represent low to high connectivity (0 value represents a barrier). Patches with at least 10 ha tall (>3 m) vegetation are shown in green. Blue lines indicate clusters of at least five roe deer observations over the last 10 years (red dots). The yellow/black line shows the least-cost path to the nearest greenspace (CD1), the blue/black line to the nearest roe deer population (CD2). Note that the least-cost path does not connect to the nearest roe deer cluster in the middle, but follows a more easterly route through some low-resistance areas before crossing the canal.

was defined as the proportion built-up and paved area. In addition, we quantified the proportion of water bodies, vegetation above 3 m, and vegetation below 3 m.

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

Two cost measures were calculated using different source areas. For the first (CD1), all suitable patches with vegetation over 3 m high (trees) and minimum 10 ha in size were used as sources, assuming they contain tick hosts. For the second (CD2), the nearest known roe deer population was used as a source. To identify these populations, we collected all roe deer observations over the last 10 years (www.waarnemingen.be, data provided by Natuurpunt) and joined observations with <500 m between them. Clusters with less than five observations were discarded as they possibly represented transient individuals. Cost distances were calculated from each sampling site to the nearest roe deer observation site that belonged to a cluster (Fig. 1b). Thus, CD1 represents the inverse of connectivity to areas suitable for any host population, while CD2 represents connectivity to known populations of the main tick host. Since CD1 and CD2 were highly correlated (Pearson's rho: 0.93, $N = 22$), the first axis of a principal component analysis was used in further analyses (labelled "cost distance"), explaining 96.7% (factor loading CD1: 0.71; f.l. CD2: 0.71).

2.5. Statistical analysis

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

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

The following model restrictions were imposed, because of the limited amount of data (maximum use of twenty-two independent clusters, i.e. sampling plots) as well as the high number of tests: (1) No interaction terms among the main explanatory variables were fitted. Adding them would lead to (almost) saturated models and reduction in statistical power for each of the tests. (2) Only those variables that

were highly significant ($P < 0.01$) are considered as main results in the discussion section and abstract. A variable that explained part of the variation, though in a less significant way ($P < 0.05$), was left in the models to remove its confounder effect. (3) A stepwise backward selection procedure was used to select the best model. At each step we excluded the fixed factor with the highest non-significant P -value ($P > 0.05$), re-ran the model and examined the P -values of the fixed factors in the reduced model. Model reduction continued until only significant factors ($P < 0.05$) remained (Steyerberg, 2009). All estimates are reported as mean \pm S.E. All data management and statistical analyses were done in SAS v 9.3 (SAS Institute, Cary, North Carolina, USA).

3. Results

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

An overview of the number of trapped ticks and sampling effort per plot is presented in Table 1. *Ixodes ricinus* was by far the most abundant tick species, but its density strongly varied among plots (range 0–3.8 ticks/m²). In the highly urbanized areas within the ring road no ticks were collected, despite intensive sampling. Tick densities were higher in 2016 than 2014, and higher in Spring than in Autumn (Table 2). Variation in tick density among sampling plots was consistent over time, as indicated by correlations for annual counts (Spring 2014 vs. Spring 2016; Spearman correlation = 0.90 for larvae, 0.94 for nymphs, 0.86 for adults; all $P < 0.02$, $N = 6$ plots) and seasonal counts (Spring vs. Autumn 2016: 0.81 for larvae, 0.79 for nymphs, 0.67 for adults, $N = 21$ plots; all $P < 0.001$). Spring and Autumn densities were significantly correlated for all developmental stages (adult vs. larva: 0.49–0.61; larva vs. nymph: 0.65–0.68; nymph vs. adult: 0.88–0.92; all $P < 0.022$).

Tick densities were significantly negatively correlated with cost distance (i.e. the inverse of connectivity) as evidenced by GEE models (Table 2 and Fig. 2, all $P < 0.035$). Furthermore, for all stages, density was negatively correlated with the level of urbanization (all $P < 0.003$). While agricultural land cover was shown to be negatively correlated with larval density (-1.47 ± 0.22 Log counts/m², Z -value: -6.63 , $P = 0.0003$), it was positively associated with adult densities (0.39 ± 0.16 ; Z -value: 2.49, $P = 0.013$).

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

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

Factors explaining variation in densities of *Borrelia* infected nymphs and adults were very similar in sign and effect size to those for overall density of nymphs and adults (Table 2 and Fig. 2). Overall, the density of *Borrelia*-infected ticks (DI-N and DI-A) was higher in 2016 than in 2014, and higher in Spring than in Autumn. Connectivity and urbanization also had similar effects on infected ticks compared to overall tick densities.

When looking into the known host associations of the *Borrelia* genospecies (Table 3), most ticks were infected with mammalian genospecies (*B. afzelii*, *B. spielmanii* and *B. burgdorferi* s.s.: 193 out of 267 successfully sequenced individuals = 72%) compared to bird genospecies (*B. garinii*, *B. valaisiana*; 74 individuals). Unfortunately, the low numbers of successfully sequenced samples were too low for further analysis on pathogen communities.

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

For those sampling plots in which at least one tick was collected, the N-IP for 2016 was on average higher than for 2014, and lower in Autumn than in Spring (Table 4). Factors explaining variation in infection

Table 2

Parameter estimates (\pm empirical standard error) from GEE models on *I. ricinus* densities using negative binomial distributed residuals. Densities refer to the density of tick individuals DO-[] and density of infected individuals DI-[] for three developmental stages (larvae [L], nymphs [N] and adults [A]). In all models, the exchangeable working correlation on the level of sampling plot has been introduced.

(A)	Larvae	Nymphs	Adults		
Effect (# sample plots)	DO-L (N = 22)	DO-N (N = 22)	DI-N (N = 22)	DO-A (N = 22)	DI-A (N = 22)
Intercept	$-2.86 \pm 0.41^{***}$	$-3.75 \pm 0.44^{***}$	$-5.63 \pm 0.63^{***}$	$-5.40 \pm 0.44^{***}$	$-6.73 \pm 0.53^{***}$
Year (diff. 2014–2016)	-0.06 ± 0.83 NS	$-1.26 \pm 0.40^{***}$	$-1.16 \pm 0.29^{***}$	-0.37 ± 0.36 NS	-0.99 ± 0.61 NS
Season (diff. Autumn-Spring)	-0.80 ± 0.47 NS	$-2.13 \pm 0.32^{***}$	$-1.50 \pm 0.35^{***}$	$-1.72 \pm 0.45^{**}$	$-1.21 \pm 0.49^*$
Urbanization	$-3.08 \pm 0.28^{***}$	$-1.16 \pm 0.29^{***}$	$-1.17 \pm 0.32^{***}$	$-1.33 \pm 0.45^{**}$	$-1.59 \pm 0.55^{**}$
Surface area	0.19 ± 0.18 NS	0.16 ± 0.17 NS	0.22 ± 0.19 NS	0.16 ± 0.16 NS	0.009 ± 0.174 NS
% Agricultural	$-1.47 \pm 0.22^{***}$	0.25 ± 0.16 NS	-0.05 ± 0.22 NS	$0.39 \pm 0.16^*$	$0.71 \pm 0.20^{***}$
% Water bodies	$-0.35 \pm 0.17^*$	-0.07 ± 0.20 NS	0.27 ± 0.17 NS	0.12 ± 0.24 NS	-0.18 ± 0.21 NS
Cost distance	$-3.03 \pm 0.47^{***}$	$-2.39 \pm 0.53^{***}$	$-1.83 \pm 0.61^{**}$	$-0.87 \pm 0.41^*$	-0.75 ± 0.61 NS
Working ρ	0.71	0.13	0.050	0.010	0.029

$P < 0.001$: ***; < 0.01 : **; $P < 0.05$: *; $P > 0.05$: NS.

prevalence of nymphs were dissimilar in sign and effect size to those of adults (Fig. 3). In nymphs, larger plots (0.20 ± 0.03 , Z-value = 6.01) with lower agricultural land cover (-0.30 ± 0.07 , Z-value = -4.30) showed higher infection prevalence (all $P < 0.001$). In adults we found the reverse associations (surface area: -0.15 ± 0.07 , agriculture: 0.70 ± 0.17). Associations with surface area were driven by one important sampling plot in the rural zone ('Boshhoek'). In addition, open water cover was positively associated with the *Borrelia* prevalence (0.37 ± 0.10 , Z-value = 3.63, $P < 0.001$).

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

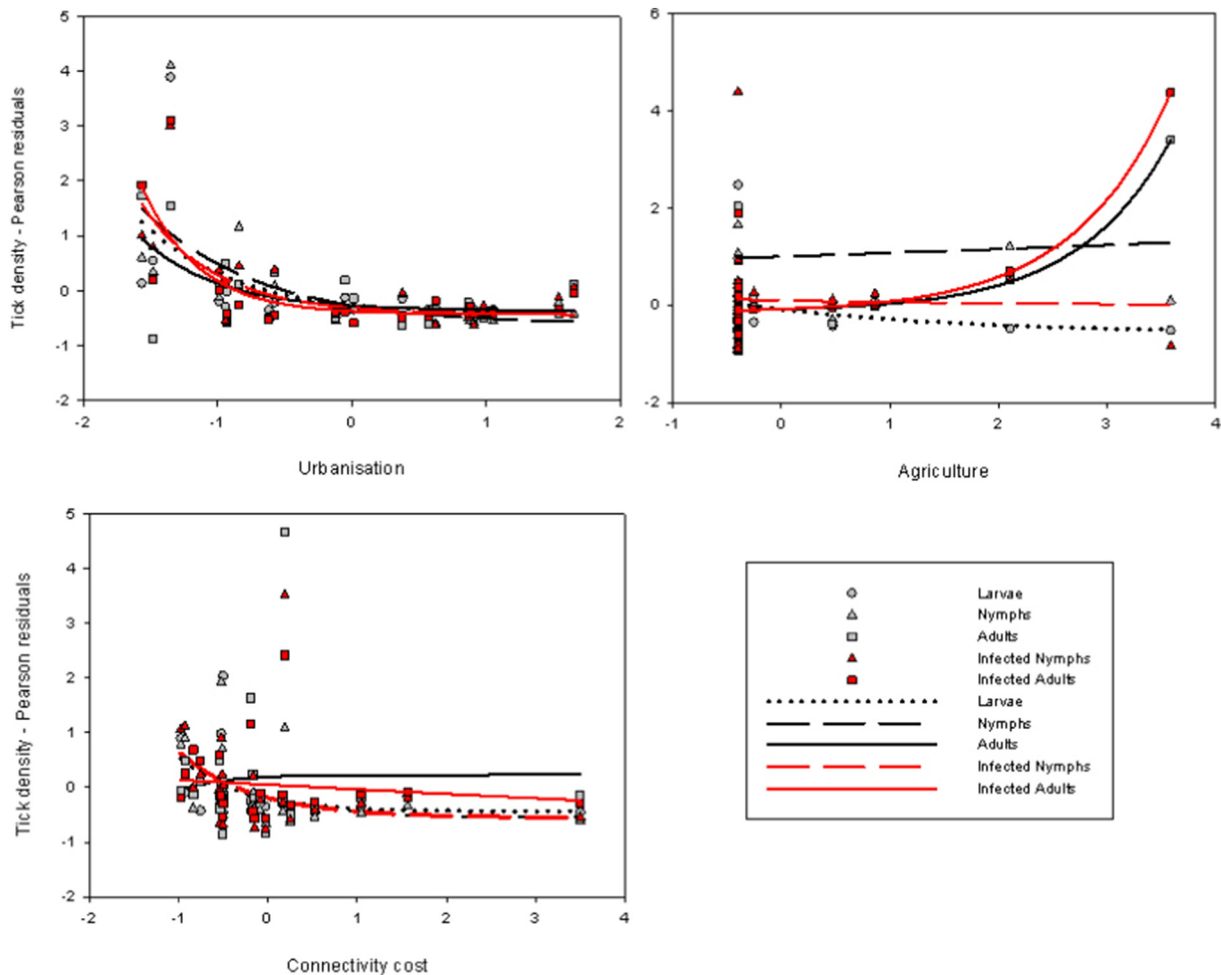


Fig. 2. Partial Pearson residuals of DO-L, -N, -A and DI-N, -A plotted against significant landscape characteristics (Table 2). Exponential curves ($a \cdot e^{bx}$) have been added based on least squares approximation.

Table 3
Borrelia burgdorferi s.l. prevalences in *Ixodes ricinus* individuals from sampling plots for which at least one tick could be screened and the distribution of avian (*B. garinii*, *B. valaisiana*) vs. mammalian (*B. afzelii*, *B. b. sensu stricto*, *B. spielmanii*) genospecies for sampling plots with at least one successfully genotyped *Borrelia* strain.

ID	Area	Urb.	<i>Borrelia b. sensu lato</i> (# qPCR screened)		Ratio (# genotyped)		<i>B. garinii</i>		<i>B. valaisiana</i>		<i>B. afzelii</i>		<i>B. b. sensu stricto</i>		<i>B. spielmanii</i>	
			N	A	N	A	N	A	N	A	N	A	N	A	N	A
1	Boshoek	ru	0.2 (708)	0.26 (91)	0.2 (106)	0.32 (20)	0.01	0.02	0.02	0.04	0.09	0.12	0.03	0.02	0.01	0.01
2	Peerdsbos	ru	0.18 (500)	0.31 (39)	0.43 (68)	0.31 (10)	0.03	0.03	0.02	0.05	0.05	0.1	0.02	0.08	0	0
3	Oude Spoorwegberm-A	ru	0 (12)	1 (6)		0.5 (6)		0.5		0	0.5		0		0	0
4	Oude Spoorwegberm-B	ru	0.08 (73)	0.6 (5)	0.5 (4)	1 (2)	0	0	0.03	0.4	0	0	0.03	0	0	0
5	Park Lint	ru	0.11 (19)	0 (1)	0.5 (2)		0.05		0		0			0.05		
6	Park Heuvelhof	ru	0.15 (13)	0.5 (2)		1 (1)		0	0.5		0		0		0	
7	George v. Raemdonckp.	ru	0.33 (3)	1 (1)		1 (1)		0		1		0		0		0
8	Rivierenhof	su	0.15 (100)	0.45 (40)	0.11 (9)	0.12 (17)	0	0	0.01	0.05	0.04	0.28	0.04	0.1	0	0
9	Domein Vogelzang	su	0 (1)	(0)												
10	Beeldenmuseumpark	su	0.18 (133)	0.1 (20)	0 (11)	0 (1)	0	0	0	0	0.05	0	0.02	0	0.02	0.05
11	FortVI	su	0.21 (19)	0.5 (2)	0 (2)	1 (1)	0	0.5	0	0	0.05	0	0.05	0	0	0
12	ParkSorghvliedt	su	0.07 (28)	0.2 (5)	0 (2)	1 (1)	0	0	0	0.2	0	0	0.04	0	0.04	0
14	Boekenbergpark	su	0 (6)	0 (2)												
17	Steytelinckpark	su	0.11 (9)	0 (1)												
18	Leeuwerikpark	su	0.67 (3)	0.5 (2)	0.5 (2)	0 (1)	0	0	0.33	0	0.33	0.5	0	0	0	0

In bold: prevalence for tick batches (# qPCR screened or # genotyped) ≥ 10 individuals.

N: nymph; A: adult.

Ratio: avian genospecies (*B. garinii* + *B. valaisiana*) over mammalian genospecies (*B. afzelii* + *B. b. sensu stricto* + *B. spielmanii*).

4. Discussion

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

4.1. Density of (infected) ticks

Despite the large number of studies documenting the presence of ticks in urban and suburban areas, most of these have not quantified variation in tick abundance (e.g. studies collecting a fixed amount of ticks for pathogen detection, such as Tappe et al., 2014) and/or focus only on sites with ticks known to be present (e.g. Junttila et al., 1999) or were based on small samples per location (e.g. Hansford et al., 2017) or a limited number of sites (e.g. Cekanac et al., 2010;

Table 4

Parameter estimates (\pm empirical standard error) from the GEE's that model the *Borrelia* prevalence for *I. ricinus* nymphs (N-IP) and *I. ricinus* adults (A-IP). Binomial distribution for residuals has been used as well as the exchangeable working correlation on the level of sampling plot.

Effect	N-IP	A-IP
	N = 14	N = 14
Intercept	-1.65 \pm 0.09***	-0.72 \pm 0.25**
Year (diff. 2014–2016)	-0.88 \pm 0.26***	-0.68 \pm 0.48 NS
Season (diff. Autumn–Spring)	-0.30 \pm 0.08***	0.38 \pm 0.66 NS
Urbanization	0.06 \pm 0.16 NS	0.04 \pm 0.55 NS
Surface	0.20 \pm 0.03***	-0.15 \pm 0.07*
% Agricultural	-0.30 \pm 0.07***	0.70 \pm 0.17***
% Water bodies	0.20 \pm 0.15 NS	0.37 \pm 0.10***
Cost distance	0.11 \pm 0.33 NS	-1.64 \pm 1.42 NS
Working ρ	-0.50	-0.50

$P < 0.001$: ***; < 0.01 : **; $P < 0.05$: *, $P > 0.05$: NS.

Kazimirova et al., 2016; Nelson et al., 2015). Other studies have reported on variation in tick abundance among different habitat types, but without taking into account the surrounding landscape (e.g. Hornok et al., 2014; Krstic et al., 2016; Maetzel et al., 2005; Rosa et al., 2018). Some of these studies suggest similar trends as in our study with lower abundance or presence of ticks in urban parks or other green spaces close to urban centers (e.g. Cekanac et al., 2010; Hansford et al., 2017; Maetzel et al., 2005; Oechlin et al., 2017; Pangracova et al., 2013), but without further quantitative details or analysis on the driving landscape characteristics. In a study including urban, agricultural and natural areas in five countries, Rosa et al. (2018) concluded that there was no significant difference among these three habitats when accounting for variation in Normalized Difference Vegetation Index (NDVI) and precipitation; however, it should be noted that several of the included urban parks were of large size and/or associated with small urban centers compared to our study.

The significant effects of urbanization and connectivity on *I. ricinus* density in our study can be readily explained by this tick's biology. The presence of larger mammals (roe deer, foxes, wild boars and others) on which adult developmental stages feed and copulate, is one of the main conditions for viable *I. ricinus* populations (Kilpatrick et al., 2017; Rizzoli et al., 2014). As one engorged fertilized adult female will give rise to >2000 larvae (Balashov, 1972; Gray, 1991; Gray, 1998), even a brief stop-over of an infested end host can initiate or boost a local *I. ricinus* population. Urban areas, and in particular city centers, typically have reduced diversity of wildlife (Aronson et al., 2016; Nielsen et al., 2014) although densities of so-called urban adapters may be as high or even higher than in rural areas (Mackenstedt et al., 2015; Rizzoli et al., 2014). At least part of the reason for the loss of species is the fragmented habitat and high resistance of the urban matrix for wildlife movement, leading to strong isolation of these areas from each other and from the putative source areas in rural areas outside the city (e.g. Braaker et al., 2017; Nielsen et al., 2014; Verbeylen et al., 2003). Thus, areas characterized by low connectivity (high cost distance value) such as the highly urbanized areas within the Antwerp ring road, may still hold populations of smaller and/or more mobile hosts such as rodents or birds, but not of larger animals such as roe deer that are major hosts of adults.

Our initial aim in this study was to discriminate between the importance of local connectivity (i.e. to nearby green spaces) and connectivity to major forested areas with known important host populations, i.e. roe deer. The first measure would then reflect the importance of intra-urban host populations (typically small and medium-sized mammals

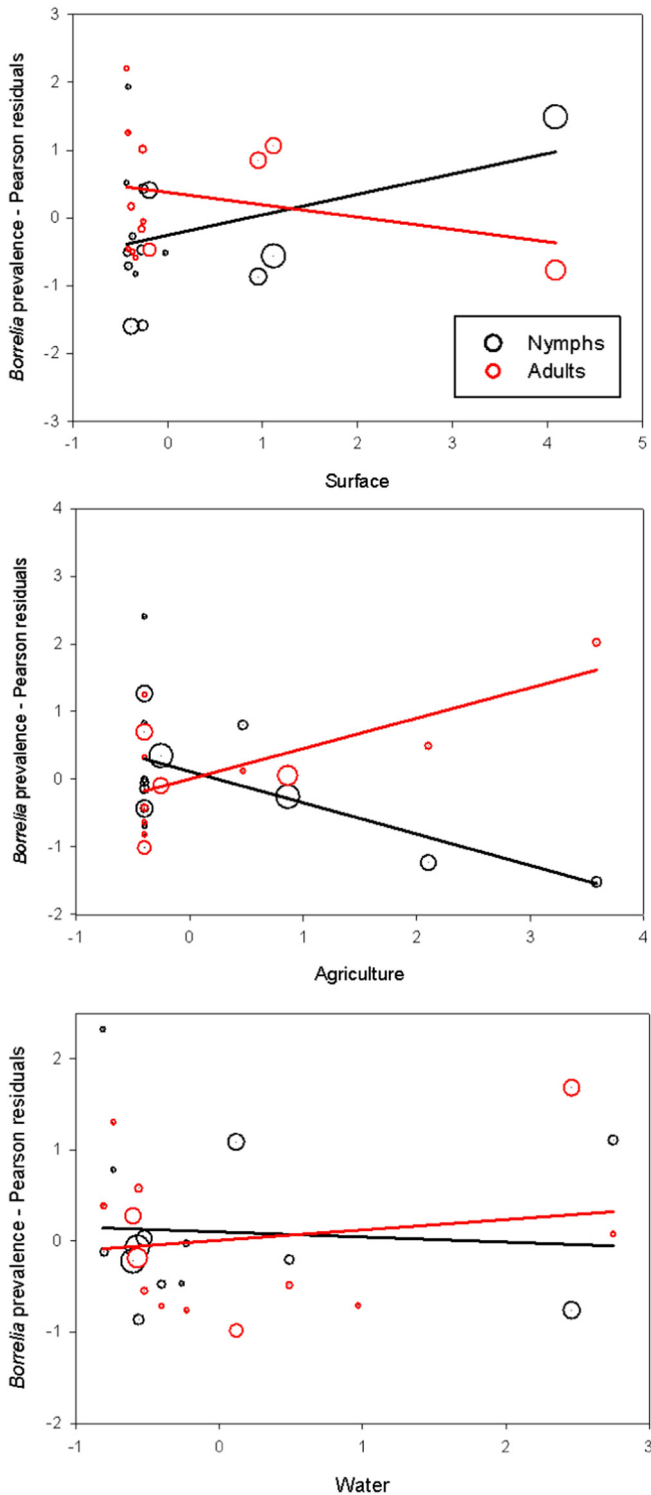


Fig. 3. Partial Pearson residuals N-IP and A-IP plotted against landscape characteristics (Table 4). Bubble size corresponds to the number of ticks collected (range adults: 1–91, range nymphs: 1–708). Linear curves ($a \cdot x$) have been added based on least squares approximation.

and birds) and/or the importance of such urban green spaces as stepping stones for larger dispersing hosts, such as occasional movements of roe deer or foxes into the (sub-)urban area. However, since the two connectivity measures proved to be more strongly correlated than we anticipated, we were unable to make this distinction. The strong correlation indicates that green spaces close to the urban center are not only far from peripheral source areas, but also mutually strongly

isolated. In contrast, suburban green spaces are closer to the periphery but also appear to have more interconnections between them. This pattern clearly reflects the lack of extensive green corridors into the city center. Other metropolitan areas with a different layout of green spaces may provide better opportunities to disentangle the effects of local versus long-distance connectivity.

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

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

In areas where ticks were present, the *Borrelia* infection rates did not show any association with degree of urbanization. This is in line with previous studies showing infection rates in urban areas to be comparable to those outside the city (cf. Rizzoli et al., 2014). Larger plots tended to have significantly higher infection prevalence, but this association was driven by a single rural area ('Boshoek'). Associations with prevalence (including agricultural land and water cover) are driven by the spatial differences in exposure levels, susceptibility, or infectiousness within and between host and vector species populations. In addition, local tick and host birth and mortality rates can be affected by the pathogen itself, resulting in prevalence differences. Currently we lack knowledge on local host communities, their population dynamics and host species interactions with *Borrelia*, making the explanation of the observed patterns very speculative.

Similar proportions of avian and mammalian *Borrelia* genospecies were found in suburban and rural areas. Even though sample sizes are too limited to warrant further analyses, the data do not support the idea that ticks in the most urban areas depend more strongly on avian versus mammalian hosts. Furthermore, the comparatively high prevalence of mammalian genospecies such as *Borrelia afzelii* suggests that even small suburban green spaces are capable of supporting enzootic cycles in local mammal populations, despite the overall low tick abundance. We cannot rule out the possibility that such cycles are supported at least in part by other tick species, notably the burrow-dwelling *Ixodes hexagonus* living on hedgehogs and competent for transmitting mammalian *Borrelia* genospecies as well (Jahfari et al., 2017). Besides *Borrelia*, we traced several rarer pathogenic agents in questing ticks.

Although poorly studied, exposure to and possibly also infection with multiple tick-borne pathogens in humans in this area seems to be the rule rather than the exception (Heylen et al., 2017).

4.3. Concluding remarks

We showed that landscape connectivity and urbanization significantly influence the variation in tick abundances (DO-L, -N, -A) and hence the *Borrelia*-exposure to humans (DI-N and -A) along an urbanization gradient. Tick densities are strongly reduced with increasing urbanization and distance from source areas. However, *Borrelia* prevalences are comparable along the urban-rural gradient, and even moderately isolated suburban green spaces may support enzootic cycles of different *Borrelia* genospecies. Our results suggest that the risk of tick-borne infections should be taken into account when designing green spaces in urban areas. In particular, improving connectivity for large propagation hosts such as roe deer, and/or creating additional habitat for these species in proximity to urban areas, may lead to a higher risk in urban areas. Our study also highlights that urban and suburban areas should be included in surveillance for tick-borne diseases and public health campaigns (Uspensky, 2014) because, even though tick densities may be low, tick infection prevalence and the risk of human contact are high. Still, substantial variation in tick density remains unexplained. Future research needs to include host surveys as well as more detailed habitat characteristics and specific resources (e.g. bird feeders,

waste) that affect host population densities. Understanding the mechanisms how spatial factors affect pathogen risk in urban areas also requires more detailed knowledge on how wildlife presence and movements respond to green space characteristics such as connectivity, habitat quality and human disturbance.

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Credit author statement

DH and EM conceived the ideas and designed methodology; RL, FM and HS collected the data; DH, RL and FA analyzed the data; DH and EM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. The authors have no conflict of interest to declare.

Appendix 1. Pathogen prevalences in *Ixodes ricinus* individuals from the twenty-two sampling plots

ID	Area	Urb. ^a	# ticks screened		<i>B. burgdorferi</i> s.l.		<i>A. phagocytophilum</i>		'Ca <i>N. mikurensis</i> '		<i>Babesia</i> sp.		<i>B. miyamotoi</i>	
			N	A	N	A	N	A	N	A	N	A	N	A
1	Boshoek	ru	708	91	0.2	0.26	0.02	0.04	0.03	0.1	0.01	0.02	0.02	0.02
2	Peerdsbos	ru	500	39	0.18	0.31	0.01	0.05	0.02	0	0.02	0.03	0.01	0
3	Oude Spoorwegberm-A	ru	12	6	0	1	0	0	0	0	0	0	0	0
4	Oude Spoorwegberm-B	ru	73	5	0.08	0.6	0.01	0	0	0	0	0	0.01	0
5	Park Lint	ru	19	1	0.11	0	0	1	0	0	0	0	0	0
6	Park Heuvelhof	ru	13	2	0.15	0.5	0	0	0	0	0	0	0	0
7	George v. Raemdonckpark	ru	3	1	0.33	1	0	0	0	0	0	0	0	0
8	Rivierenhof	su	100	40	0.15	0.45	0.02	0.03	0	0	0	0	0.02	0.1
9	Domein Vogelzang	su	1	0	0	0	0	0	0	0	0	0	0	0
10	Beeldenmuseumpark	su	133	20	0.18	0.1	0.02	0	0	0	0	0	0.02	0
11	FortVI	su	19	2	0.21	0.5	0	0	0	0	0	0	0	0
12	ParkSorghvliedt	su	28	5	0.07	0.2	0	0	0	0.2	0	0	0.04	0
13	Wolvenberg	su	0	0	0	0	0	0	0	0	0	0	0	0
14	Boekenbergpark	su	6	2	0	0	0	0	0	0	0	0	0	0
15	Brilschanspark	su	0	0	0	0	0	0	0	0	0	0	0	0
16	Mastvest	su	0	0	0	0	0	0	0	0	0	0	0	0
17	Steytelinckpark	su	9	1	0.11	0	0	0	0	0	0	0	0	0
18	Leeuwerikpark	su	3	2	0.67	0.5	0	0	0	0	0	0	0	0
19	Park Spoor Noord	u	0	0	0	0	0	0	0	0	0	0	0	0
20	Stadspark	u	0	0	0	0	0	0	0	0	0	0	0	0
21	Hof van Leysen	u	0	0	0	0	0	0	0	0	0	0	0	0
22	Koning Albertpark	u	0	0	0	0	0	0	0	0	0	0	0	0
	Overall		1627	218	0.178	0.326	0.017	0.037	0.017	0.046	0.006	0.014	0.015	0.028

In bold: prevalence for tick batches ≥ 10 individuals.

N: nymph; A: adult.

^a Urbanisation level: rural (ru), suburban (su), urban (u).

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