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Impact of green space connectivity on urban tick presence, density and *Borrelia* infected ticks in different habitats and seasons in three cities in southern England

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Abstract

Understanding the effects of local habitat and wider landscape connectivity factors on tick presence, nymph density and *Borrelia* species (spp.) prevalence in the tick population is important for identifying the public health risk from Lyme borreliosis. This multi-city study collected data in three southern England cities (Bath, Bristol, and Southampton) during spring, summer, and autumn in 2017. Focusing specifically on urban green space used for recreation which were clearly in urbanised areas, 72 locations were sampled. Additionally, geospatial datasets on urban green space coverage within 250 m and 1 km of sampling points, as well as distance to woodland were incorporated into statistical models. Distance to woodland was negatively associated with tick presence and nymph density, particularly during spring and summer. Furthermore, we observed an interaction effect

between habitat and season for tick presence and nymph density, with woodland habitat having greater tick presence and nymph density during spring. *Borrelia* spp. infected *Ixodes ricinus* were found in woodland, woodland edge and under canopy habitats in Bath and Southampton. Overall *Borrelia* spp. prevalence in nymphs was 2.8%, similar to wider UK studies assessing prevalence in *Ixodes ricinus* in rural areas. Bird-related *Borrelia* genospecies dominated across sites, suggesting bird reservoir hosts may be important in urban green space settings for feeding and infecting ticks. Whilst overall density of infected nymphs across the three cities was low (0.03 per 100 m²), risk should be further investigated by incorporating data on tick bites acquired in urban settings, and subsequent Lyme borreliosis transmission.

Keywords

connectivity, *Borrelia*, ticks, *Ixodes ricinus*, Lyme borreliosis, public health

Introduction

Urban landscapes are supported by green infrastructure which delivers a multitude of ecosystem services, from improving health and wellbeing to carbon storage and heat reduction (Office for National Statistics, 2018). The public health benefits of access to urban green space have long been recognised and support increased physical activity, improvement to mental health and wellbeing and provide opportunity for social contact (Lee et al., 2015; Wheeler et al., 2015). There is also evidence for increasing urban green space to reduce the urban heat island effect and reduce CO² emissions along with levels of noise and pollution (Kruizse et al., 2019). These important spaces have the potential to benefit a wide range of public health goals and as a result, plans to increase urban green space, along with its quality and accessibility are already being executed on a national and international scale (Defra, 2011; WHO, 2017). Tick species important to public health, however, are increasingly being reported within urban green space habitat (Rizzoli et al., 2014; Hansford et al., 2022). This presents not only a potential public health challenge but could also undermine the public health gains to be harnessed from urban green space.

Urbanisation is likely to have an impact on tick populations and pathogen transmission dynamics through modification of habitat and changes in wildlife host composition (Pfäffle et al., 2013; Uspensky, 2014). Green urban infrastructure includes urban forests, open green space, and green corridors (for example wildflower verges and hedgerows), which can form a network supporting diverse plant species and migration of wildlife from surrounding rural habitat. This network can provide transient or stable habitats for biodiversity in urban areas (Angold et al., 2006) and wildlife movement can be facilitated, particularly if nearby home ranges for wildlife exist (Evans et al., 2010). Other factors also influence wildlife movements across urban landscapes, including spatial connectivity, habitat quality and resource pressure in surrounding habitat (Evans et al., 2010). These urban green networks can be utilised by a wide range of important tick maintenance hosts (see Rizzoli et al. 2014 for full review), including several deer species found in the UK, primarily roe (*Capreolus capreolus*), muntjac (*Muntiacus reevesi*) and sometimes red deer (*Cervus elaphus*) (The British Deer Society, 2021; The Deer Initiative, 2011). Small mammals, including bank voles (*Myodes glareolus*), wood mice (*Apodemus sylvaticus*) and yellow-necked mice (*Apodemus flavicollis*), as well as ground foraging birds such as blackbirds (*Turdus merula*) are important for feeding and infecting juvenile tick life stages with *Borrelia* spp. (Cull et al., 2017; Dubska et al., 2009; Heylen et al., 2019; Krawczyk et al., 2020; Kurtenbach et al., 1998) and are also likely play a role in sustaining and moving ticks around urban green space (Baker et al., 2003; Bush et al., 2012; Evans et al., 2009; Fuentes-Montemayor et al., 2020; Perez et al., 2016). Although the above host groups may differ in urban habitat use and urban resilience, they all benefit from high quality woodland habitat, which can provide food and shelter, and greater connectivity across the landscape (Fuentes-Montemayor et al., 2020; Heylen et al., 2019; Hinsley et al., 1996; Lovari et al., 2017; Morellet et al., 2013).

Ixodes ricinus is a key vector of a wide range of human and animal pathogens found across the northern temperate zone (Gray, 2002). Along with increased distribution of this tick species in recent decades (Medlock et al, 2013), increased tick-borne disease incidence has been reported across Europe (Kilpatrick and Randolph, 2012; Sprong et al., 2018). Whether these changes have been

driven by climate change, habitat modification, vector adaptation, wildlife host population dynamics or human behaviour (or a combination of these factors which have not yet been quantified) remains an important debate (Gray et al., 2021; Kilpatrick et al., 2017; Medlock et al., 2013). Increased attention and research on tick distribution has also revealed that across Europe, urban green space provides suitable habitat (microclimate) for the off-host survival of ticks (Hansford et al., 2022; Rizzoli et al., 2014; Uspensky, 2014). The presence of *I. ricinus* in urban green space is not necessarily a new phenomenon (Hansford et al., 2022; Rizzoli et al., 2014; Uspensky, 2014), but tick encounters may have increased over time, particularly in recent years following initiatives to increase urban green space and its use by members of the public (Office for National Statistics, 2018).

There are clear questions yet to be answered on the drivers of tick presence, density, and seasonal activity of ticks within urban green space, as well as the prevalence of important human pathogens and potential for tick-human contact. A better understanding of these drivers could contribute to better urban green space planning to maximise health benefits and minimise the risks associated with tick-borne disease exposure. The aims of this study were to investigate Lyme borreliosis risk factors including questing tick presence, nymphal density and *Borrelia* spp. prevalence in ticks collected in urban green space across three cities in southern England. This multi-city study incorporated different habitat types within the same green space patch, covering areas strictly within city boundaries. It also captured data in different seasons (once during spring, summer, and autumn) to identify potentially higher risk periods for tick activity. Additionally, it investigated landscape factors, including the proportion of green space surrounding survey locations (a proxy for habitat connectivity for tick and *Borrelia* spp. hosts) and the distance to nearest woodland habitat (key sources of ticks and/or tick hosts). Due to the importance of woodland and woodland edge in supporting ticks and tick hosts, we expected the presence of these habitats within survey locations would increase tick presence and nymph density compared to patches without these key habitat types. Spring was predicted to have higher nymph density compared to other seasons, due to optimal microclimatic conditions for tick activity (Gilbert et al., 2021; Humair and Gern, 2000).

Borrelia spp. prevalence was expected to vary by season, being higher during spring and summer as reported previously in a study in a small city in southern England (Hansford et al., 2017). Finally, it was predicted that a higher proportion of green space and shorter distance to woodland will facilitate tick presence, density and *Borrelia* spp. prevalence due to increased accessibility for key tick/*Borrelia* spp. hosts (Heylen et al., 2019).

Methods

City selection

Three cities in southern England (Bath, Bristol and Southampton) with records of *I. ricinus* reported through national surveillance (Public Health England, 2017a), evidence of laboratory-confirmed cases of Lyme borreliosis (Public Health England, 2017b), local implementation of national policy on urban green space management (NPPF, 2012; PPG 17, 2002) and accessible spatial urban green space typology datasets were chosen for this study. Bath has an estimated population of 88,859 (Bath & North East Somerset Council, 2015a) and a wide range of green infrastructure exists (e.g., parks, open spaces, woodlands, allotments, private gardens etc) which makes up approximately 950 ha of urban green space (Bath & North East Somerset Council, 2015a; Table 1). Bristol is the 10th largest city in the UK with a population of 442,500. The city has over 100 conservation and wildlife areas and almost 100 parks and gardens (Bristol City Council, 2015, 2008). There are 1500 ha of accessible urban green space used specifically for recreation in Bristol (Table 1; Bristol City Council, 2008) which are visited 25 million times annually (Bristol City Council, 2015). Southampton is a port city with a population of 228,600. A wide range of urban green spaces exist across the city (Table 1), which cover approximately 1100 ha of land (Southampton City Council, 2008).

Table 1. Green space typology categories determined by local authorities in Bath, Bristol and Southampton based upon local interpretation of national policy (PPG 17, 2002) and locally derived priorities (Bath & North East Somerset Council, 2015a; Bristol City Council, 2008; Southampton City Council, 2008). Those in bold were included in this study.

Bath	Bristol	Southampton
Allotments	Active Sports Space	Allotments
Amenity Green Space	Children & Young People's	Amenity Green Space
Community Growing Space	Space	Community Gardens
Churchyards & Cemeteries	Formal Green Space	Formal Parks & Gardens
Education (e.g., sports)	Informal Green Space	Green Corridors
Historic Parks & Gardens	Natural Green Space	Natural & semi-natural
Natural Green Space		Green Space
Outdoor sport		Outdoor Sports
Parks & Recreation		Provision for Children &
Play Space		Teenagers
		Urban Farms

GIS datasets and survey location

Multiple datasets were used to randomly select survey locations across Bath, Bristol, and Southampton. Survey locations had to fall within the boundary of each city (Office for National Statistics, 2015). This dataset is based upon 2011 census data, with the boundary following the built-up area, rather than administrative boundaries which can often include the surrounding rural area. To identify potential survey locations, local typology of urban green space spatial datasets were obtained from relevant local authorities (datasets were supplied by Bath and North East Somerset Council, Bristol City Council and Southampton City Council). Although all datasets were based upon Planning Policy Guidance 17 classifications (PPG 17, 2002), typology categories differed across cities. As a result, each typology was reviewed (Table 1) and those relevant for this study were restricted to publicly accessible spaces used specifically for recreation and managed by the authority. Areas outside of local authority management e.g., owned by other organisations, or privately owned were excluded.

To further standardise location selection across the three cities, Urban Atlas (2012), a freely accessible dataset that provides details of typology across urban areas in Europe was used. Three of

the 28 typologies included in Urban Atlas (2012) were considered relevant; green urban areas (publicly accessible spaces such as gardens, parks and some natural or woodland areas used for recreational purposes), forest and pasture. All Urban Atlas (2012) green urban areas, forest or pasture also identified in local authority spatial datasets as a publicly accessible green space for recreational use were allocated with a number. Half of all potential survey locations were then randomly selected for sampling using random number generation (Figure 1). 14 locations were randomly selected in Bath, 38 in Bristol and 20 in Southampton.

Habitat classification

Habitat was investigated as an explanatory variable, with four broad habitat types used: open, under canopy, woodland and woodland edge. Up to four habitat types were sampled for ticks at each randomly selected location. "Under canopy" habitat was represented by any space under canopy that could not be classed as woodland or woodland edge e.g., single, or small groups of trees within open habitat. "Open" habitat was represented by any space not under canopy and included areas with both long and short grass. Woodland habitat was classed based upon dense groupings of trees of at least 20 m² that formed a continuous canopy and woodland edge was the edge of woodland, also known as ecotonal habitat, where woodland transitions to another adjacent habitat.

Connectivity data

Two landscape connectivity metrics were generated for each survey location. The proportion of green space within an area could be a useful proxy for accessibility for wildlife, and thus a potential predictor for tick presence, nymphal density or *Borrelia* spp. prevalence (Heylen et al, 2019). UKCEH Land Cover Map 2015 (Rowland et al., 2017) was combined with OS Greenspace MasterMap (using the dissolve tool in ArcGIS Pro) to generate a comprehensive map of green space coverage. To determine the proportion of green space around survey locations, the mean XY coordinates of all transects at a survey location were used as a centroid to generate circular buffers at 250 m and 1 km radius. A buffer of 250 m was chosen to assess green space connectivity within the immediate

vicinity of a sampling location, whereas the 1 km buffer was for wider landscape connectivity. Using ArcGIS Pro, buffers were clipped, and the tabulate intersection tool used to generate the percentage cover of overall green space surrounding each survey location. Distance to woodland was assessed as an indication of connectivity to habitat that is a potential source of ticks or tick hosts. Distance to woodland was generated using Forestry Commission Open Data, specifically the National Forest Inventory Woodland England 2017 dataset (Forestry Commission, 2019). The 'near' tool was used to determine the shortest distance to woodland from the mean XY coordinates of each group of transects per survey location. Locations mapped in this dataset include woodland or forest > 0.5 ha with 20% canopy cover (or the potential to reach 20%) with a minimum width of 20 m (Forestry Commission, 2019).

Tick collection

Questing ticks were collected by flagging a 1 x 1 m² piece of brushed cotton cloth over the vegetation (Milne, 1943) to obtain a density estimate.. Where possible, 10 x 10 m² transects were sampled within each habitat type within each randomly selected location during each seasonal survey e.g., up to 40 x 10 m² transects in locations with all four habitat types were sampled during spring. Transects were completed on low lying vegetation and were spread out across the survey location. Data were collected during spring (1st-11th May 2017), summer (4th-25th July 2017) and autumn (26th September – 5th October 2017) on dry days between 10am and 3pm. The majority of open habitat included in the study was managed to maintain short vegetation which is not favourable for tick survival (Cekanac et al., 2010; Kowalec et al., 2017; Maetzel et al., 2005; Medlock et al., 2012). Open habitat was, therefore, surveyed during the spring period only, when nymph density is highest (Hansford et al., 2017). Under canopy, woodland and woodland edge were surveyed during spring, summer, and autumn. Location number, transect number (1-10), habitat (open, under canopy, woodland, woodland edge), number of ticks of each life stage (males, females,

nymphs, larvae) and season (spring, summer, autumn) were captured for each transect. Ticks were collected into Eppendorf tubes and stored at -80°C until further analysis.

Ticks were identified to species level based on morphology (Estrada-Peña et al., 2017; Hillyard, 1996) and nymphs and adults were placed into individual PCR tubes. Any larvae collected from the same transect were placed into the same tube in groups of up to 10 for pooled DNA extraction and *Borrelia* spp. testing. DNA from individual nymphs and adults was extracted using ammonium hydroxide (NH₄OH) (Cull et al., 2021). DNA extracts were tested for the presence of *Borrelia* spp. DNA using a pan-*Borrelia* spp. qPCR targeting the 16S rRNA gene. Positive samples were sequenced to determine *Borrelia* genospecies using the 5S-23S rRNA intergenic spacer region as described in Hansford et al., (2015).

Statistical analysis

All statistical analyses were carried out in R version 4.1.1 (R Core Development Team, 2021). The effects of city, habitat type, season, proportion of green space and distance to woodland on tick presence, nymphal density, *Borrelia* spp. prevalence and the density of *Borrelia* spp. infected nymphs (DIN) were investigated using generalized linear mixed models (GLMM). Explanatory variables were assessed for collinearity by calculating variance inflation factors (VIF) using the car package (Fox and Sanford, 2019). Any variables with a VIF higher than 3 must be discarded from the models (Zuur et al., 2009), but none of the variables included had a VIF above 2. Model selection was performed using the *dredge* function from the MuMIn package (Barton, 2020) and models with the lowest AICc (Akaike information criterion) were selected as the 'best-fit' model (Brewer et al., 2016; Zuur et al., 2009). Significance of fixed effects in these best-fit models was determined by comparing two nested models, with and without the factor of interest, using likelihood ratio tests (lmtest; Zeileis and Hothorn, 2002). If a model revealed significant effects or significant interaction effects, post-hoc Tukey tests (multcomp; Hothorn et al., 2008; emmeans; Lenth, 2020) were used to assess pairwise comparisons between categorical variables. Interactions between categorical and

continuous variables were further investigated using `ggpredict`, to plot the estimated response variable for each of the categories/levels. Stratified models (GLMMs) were also run across categories/levels where there was evidence of interaction. Violation of model assumptions (overdispersion, zero-inflation) were checked using the `DHARMA` package (Hartig, 2019). Due to a small number of ticks collected from open habitat, and lack of *Borrelia* spp. detection in these ticks, open habitat was removed from further analysis. Statistically significant terms ($p < 0.05$) are highlighted and 95% confidence intervals (CI) are presented which were calculated using the `DescTools` package (Signorell, 2021).

Questing tick presence

A GLMM with a binomial error and logit link function was used to investigate tick presence (absence / presence; all tick stages) in relation to city, habitat type, season, proportion of green space within 250 m and distance to woodland. The interaction between habitat and season, proportion of green space or distance to woodland, between season and proportion of green space or distance to woodland, and between proportion of green space and distance to woodland were also investigated. Survey location was included in the model (and all subsequent models) as a random effect, to account for the non-independence of observations from the same area (Harrison, 2014). To further investigate connectivity at a wider scale, the same model described above was repeated, but the variable 'proportion of green space within 250 m' was replaced with 'proportion of green space within 1 km'.

Questing nymph density

A GLMM with a Poisson error and log link function was used to investigate nymph density, as there was no evidence of overdispersion. The number of nymphs collected per 10 m² was used as the response variable and city, habitat type, season, proportion of green space within 250 m and distance to woodland were included as fixed effects. The interaction between habitat and season, proportion of green space or distance to woodland, between season and proportion of green space

or distance to woodland, and between proportion of green space and distance to woodland were also investigated. Only nymphs were used for density analysis, as they have the most significant impact on human health (Kilpatrick et al., 2017) and low numbers of adults were collected. To further investigate connectivity at a wider scale, the same model described above was repeated, but the variable 'proportion of green space within 250 m' was replaced with a new variable 'proportion of green space within 1 km'.

Borrelia spp. infection in questing ticks and the density of infected nymphs

A GLMM with a binomial error and logit link function was used to investigate *Borrelia* spp. infection in individual ticks in relation to city, habitat, season, tick life stage (adults vs nymphs), proportion of green space within 250 m and distance to woodland. There was insufficient data to investigate the effect of interaction terms on *Borrelia* spp. prevalence. To further investigate connectivity at a wider scale, the same model described above was repeated, but the variable 'proportion of green space within 250 m' was replaced with 'proportion of green space within 1 km'. A GLMM with a Poisson error and log link function was used to investigate the influence of city, habitat type, season, the proportion of green space within 250 m and the distance to woodland on the density of infected nymphs (DIN; one estimated DIN per randomly selected survey location per season). There was insufficient data to investigate DIN at the transect level or possible interaction effects. Furthermore, DIN models did not converge because of a lack of infected ticks in Bristol, under canopy habitat or in autumn. Analysis of DIN was consequently excluded and a model investigating the effect of green space proportion within 1 km was not attempted.

Results

Summary

Across the three cities, 878 ticks (all identified as *I. ricinus*) were collected from 27 (37.5%) of 72 locations during spring, summer, and autumn 2017, represented by 23 (2.6%) males, 23 (2.6%) females, 464 (52.8%) nymphs and 368 (41.9%) larvae (Table 2; Supplementary). Only two larvae were detected during spring; the rest were found during summer and autumn. Tick presence (one or more of any tick life stage) was detected on 205 (5.4%) of 3810 10 m² transects and were collected in all habitat types surveyed (Figure 2). All life stages were detected in Bath and Southampton, but only adults and nymphs were detected in Bristol. *Borrelia* spp. infected ticks were detected at 10 (13.9%) of 72 locations, with positive ticks found in under canopy, woodland, and woodland edge habitat in Bath and Southampton. Infected ticks were not detected in Bristol, or from open habitat in any of the cities surveyed (Table 3). *Borrelia* spp. prevalence in nymphs was 2.8% overall (n=13/457; 95% CI 1.4-4.8; Table 3). Overall density of nymphs across all cities was 1.22 (95% CI 0.91-1.52) per 100 m², and DIN was 0.03 (95% CI 0.02-0.06) per 100 m².

Table 2. Ticks collected during spring, summer, and autumn 2017 in the cities of Bath, Bristol, and Southampton, showing the area (m²) sampled in each habitat, the total collected and mean density per 100 m² and 95% confidence intervals of each life stage. ♂ = male, ♀ = female, N = nymph

Habitat	Total area m ²	♂ Total; mean per 100 m ² (95% CI)	♀ Total; mean per 100 m ² (95% CI)	N Total; mean per 100 m ² (95% CI)	Total ticks (incl. larvae); mean per 100 m ² excl. larvae (95% CI)
Open	6070	0 0 (0)	0 0 (0)	5 0.08 (0-0.17)	5 0.08 (0-0.17)
Under canopy	13400	2 0.02 (0-0.4)	1 0.01 (0-0.02)	15 0.11 (0.05-0.18)	69 0.13 (0.01-0.20)

Woodland	9990	18 0.18 (0.09-0.27)	17 0.17 0.06-0.258	395 3.95 (2.82-5.09)	742 4.30 (3.14-5.47)
Woodland edge	8640	3 0.03 (0-0.07)	5 0.05 (0-0.12)	49 0.57 (0.37-0.76)	62 0.66 (0.45-0.87)
Total	38100	23 0.06 (0.03-0.08)	23 0.06 (0.03-0.09)	464 1.22 (0.91-1.52)	878 1.34 (1.02-1.65)

Table 3 - Ticks tested for *Borrelia* from the cities of Bath, Bristol, and Southampton, grouped overall by habitat type, and broken down into life stage with the total ticks tested, total positive and 95% confidence intervals ♂ = male, ♀ = female, N = nymph, Ba = *Borrelia afzelii*, Bg = *Borrelia garinii*, Bv = *Borrelia valaisiana*, Ut = untyped, N/A= none tested

Habitat	<i>Borrelia</i> spp. positive ♂/ tested; % (95% CI)	<i>Borrelia</i> spp. positive ♀/ tested; % (95% CI)	<i>Borrelia</i> spp. positive N/ tested; % (95% CI)	<i>Borrelia</i> spp. positive total / tested; % (95% CI)	Genospecies			
					Ba	Bg	Bv	Ut
Open	N/A	N/A	0/5 0% (0-52.2)	0/5 0% (0-52.2)	0	0	0	0
Under Canopy	0/1 0% (0-97.5)	1/1 100% (2.5-100)	0/14 0% (0-23.2)	1/16 6.3% (0.2-30.2)	0	1♀	0	0
Woodland	2/18 11.1% (1.4-34.7)	4/17 23.5% (6.8-49.9)	11/390 2.8% (1.4-5.0)	17/425 4% (2.3-6.3)	1♀, 2N	1♀	2♀, 6N	2♂, 3N
Woodland Edge	1/3 33.3% (0.8-90.6)	2/4 50% (6.8-93.2)	2/48 4.2% (0.50-14.3)	5/55 9.1% (3.0-20.0)	0	1N	1♂, 1♀	1♀, 1N
Total	3/22 13.6% (2.9-34.9)	7/22 31.8% (13.9-54.9)	13/457 2.8% (1.5-4.8)	23/501 4.6% (2.9-6.8)	1♀, 2N	2♀, 1N	1♂, 3♀, 6N	2♂, 1♀, 4N

Tick presence

The best-fit model to explain variation in tick presence included city, distance to woodland and the interaction between habitat and season. City was a significant predictor of tick presence in the model ($\chi^2 (2) = 17.81$, $p < 0.001$), with presence significantly more likely in Southampton (11.1%; $n=120/1077$) and Bath (8.6%; $n=69/799$) compared to Bristol (0.8%; $n=16/1934$; Table 4).

Table 4 - Tukey's test between fixed effects for all models. Estimates are for the first level listed in the pairwise contrasts. SE = standard error

Model	Pairwise contrasts	Estimate	SE	z	p
Tick presence (model 1)	Bath/Bristol	2.76	0.80	3.44	0.002
	Bath/Southampton	0.01	0.74	0.01	0.999
	Southampton/Bristol	2.75	0.69	3.95	<0.001
Tick presence (model 1) Spring	Woodland/under	1.28	0.39	3.22	0.004
	Woodland edge /under	1.18	0.42	2.82	0.014
	Woodland/ Woodland edge	0.10	0.30	0.34	0.940
Tick presence (model 1) Summer	Woodland/under	2.84	0.64	4.46	<0.001
	Woodland edge /under	1.05	0.71	1.49	0.296
	Woodland/ Woodland edge	1.79	0.40	4.45	<0.001
Tick presence (model 1) Autumn	Woodland/under	0.65	0.69	0.95	0.607
	Woodland edge /under	0.72	0.95	0.75	0.731
	Woodland/ Woodland edge	1.37	0.79	1.73	0.192
Nymph	Bath/Bristol	3.29	0.99	3.46	0.003

density (model 2)	Bath/Southampton	0.14	0.91	0.16	0.986
	Southampton/Bristol	3.15	0.88	3.56	0.001
Nymph density (model 2) Spring	Woodland/under	1.33	0.46	2.91	0.010
	Woodland edge /under	0.31	0.47	0.65	0.794
	Woodland/ Woodland edge	1.02	0.23	4.55	<0.001
Nymph density (model 2) Summer	Woodland/under	2.88	0.80	3.59	0.001
	Woodland edge /under	0.39	0.87	0.46	0.892
	Woodland/ Woodland edge	2.48	0.37	6.77	<0.001
Nymph density (model 2) Autumn	Woodland/under	1.62	1.11	1.46	0.313
	Woodland edge /under	0.29	1.29	0.22	0.973
	Woodland/ Woodland edge	1.91	0.75	2.53	0.031
<i>Borrelia</i> spp. prevalence (model 3)	Adults/ nymphs	2.30	0.45	5.05	<0.001

The interaction between habitat and season was a significant predictor of tick presence (χ^2 (4) = 19.47, $p = 0.001$). Woodland and woodland edge habitat were significantly more likely to have tick presence compared to under canopy habitat in spring (Table 4). Tick presence in woodland was significantly higher compared to woodland edge and under canopy habitat during the summer, and no significant differences were detected between habitats in autumn (Table 4). Distance to woodland was also a significant predictor of tick presence (χ^2 (1) = 8.67, $p = 0.003$), with decreased

distance associated with increased likelihood of tick presence. Tick presence was predicted to be less likely in green space if woodland habitat was further than 250 m away (Figure 3).

The interactions between distance to woodland and habitat ($\chi^2 (2) = 1.18$, $p = 0.556$) or distance to woodland and season ($\chi^2 (2) = 0.95$, $p = 0.621$) were not significant predictors of tick presence. The proportion of green space within 250 m was also not a significant predictor of tick presence ($\chi^2 (1) = 0.393$, $p = 0.531$), nor was its interaction with habitat ($\chi^2 (3) = 1.18$, $p = 0.757$), season ($\chi^2 (3) = 3.74$, $p = 0.291$) or distance to woodland ($\chi^2 (2) = 2.09$, $p = 0.352$). The proportion of green space within 1 km, had a borderline significant interaction effect with season ($\chi^2 (2) = 5.42$, $p = 0.066$), but was not retained in the best-fit model so was not investigated further. The interaction between the proportion of green space within 1 km and habitat ($\chi^2 (2) = 1.32$, $p = 0.515$) or the proportion of green space within 1 km and distance to woodland was also not significant ($\chi^2 (1) = 0.81$, $p = 0.368$).

Nymph density

The best-fit model to explain the variation in nymph density included city and the interactions between habitat and season, habitat and proportion of green space within 250 m, and distance to woodland and season. When replacing the proportion of green space within 250 m with the proportion of green space within 1 km, the best-fit model included city, the interactions between habitat and season, distance to woodland and season, and green space proportion within 1 km and season.

Like the proportion of positive transects, city was a significant predictor of nymph density ($\chi^2 (2) = 16.61$, $p < 0.001$), being significantly higher in Southampton (3.16 per 100 m²; 95% CI 2.16-4.16) and Bath (1.29 per 100 m²; 95% CI 0.80-1.78) compared to Bristol (0.11 per 100 m²; 0.04-0.18; Table 4). The interaction between habitat and season was a significant predictor of nymph density ($\chi^2 (4) = 21.38$, $p < 0.001$). Woodland habitat had significantly higher nymph densities compared to under canopy and woodland edge habitat during spring and summer but densities in autumn were only significantly higher in woodland compared to woodland edge (Table 4). Unlike for tick presence, the

proportion of green space within 250 m and its interaction with habitat was significant ($\chi^2 (2) = 9.48$, $p = 0.009$), being positive in woodland and woodland edge but negative in under canopy habitat (Figure 4; Supplementary). When analysing the habitats separately, however, the associations were not significant in woodland ($\chi^2 (1) = 0.50$, $p = 0.479$), woodland edge ($\chi^2 (1) = 0.73$, $p = 0.393$) or under canopy habitat ($\chi^2 (1) = 0.15$, $p = 0.670$). This interaction was not significant at 1 km ($\chi^2 (2) = 1.88$, $p = 0.390$).

The interaction between distance to woodland and season was borderline significant ($\chi^2 (2) = 5.14$, $p = 0.077$). Increasing distance to woodland was associated with lower nymph density during spring ($\chi^2 (1) = 4.70$, $p = 0.030$) and summer ($\chi^2 (1) = 4.50$, $p = 0.034$; Supplementary). There was insufficient data to investigate the effect of distance to woodland on nymph density during the autumn (the model failed to converge). The interaction between season and the proportion of green space within 1 km was borderline significant ($\chi^2 (2) = 5.87$, $p = 0.053$). Although increasing proportion of green space within 1 km was positive associated with nymph density in spring and summer (Supplementary), analysing seasons separately showed this was not significant in spring ($\chi^2 (1) = 1.90$, $p = 0.168$) or summer ($\chi^2 (1) = 2.58$, $p = 0.108$) respectively. There was insufficient data to investigate the effect of proportion of green space within 1 km on nymph density during the autumn (the model failed to converge). Interaction effects between habitat and distance to woodland ($\chi^2 (2) = 0.26$, $p = 0.878$), season and proportion of green space within 250 m ($\chi^2 (2) = 2.88$, $p = 0.237$), as well as between distance to woodland and proportion of green space were not significant ($\chi^2 (1) = 0.86$, $p = 0.353$).

Borrelia spp. prevalence, genospecies and density of infected nymphs

Overall, 715 *I. ricinus* ticks were tested for *Borrelia* spp. (Table 3; Supplementary). Ticks were tested from 23 urban locations (Bath n=6, Bristol n=4, Southampton n=13). Infected ticks were detected from 10 locations: three in Bath and seven in Southampton. No infected ticks were detected in Bristol (n=0/24; Supplementary). Overall *Borrelia* spp. prevalence in all tick life stages (including

larvae) was 3.2% (n=23/715; 95% CI 2.1-4.8). *Borrelia* spp. prevalence was 4.6% when excluding larvae (n=23/501; 95%CI 2.9-6.8), 22.7% in adults only (n=10/44; 95% CI 11.5-37.8) and 2.8% in nymphs (n=13/457; 95% CI 1.5-4.8). None of the larvae tested positive. Infected ticks were found in all but open habitat, from three locations in Bath (locations 15, 24, and 27) where 3.0% of nymphs (n=3/101; 95% CI 0.6-8.4) and 16.7% of adults (n=3/18; 95% CI 3.6-41.4) were positive. Seven locations in Southampton had infected ticks (locations 30, 33, 38, 46, 62, 66, 73; Supplementary), with 3.0% of nymphs (n=10/334; 95% CI 1.4-5.4) and 20.6% of adults (n=7/34; 95% CI 8.7-37.9) positive. In both cities with infected nymphs, woodland edge habitat had a prevalence of 4.2% (95% CI 0.50-14.3) and woodland had a prevalence of 2.8% (95% CI 1.4-5.0; Table 3). Infected nymphs were not detected in open or under canopy habitat (Table 3).

Borrelia valaisiana accounted for 43.5% (n=10/23; 95% CI 30.6-73.2) of all *Borrelia* spp. positive ticks, followed by *Borrelia garinii* (13.0%, n=3/23; 95% CI 5.0-38.8) and *Borrelia afzelii* (13.0%, n=3/23; 95% CI 2.8-33.6; Table 3). Sequence data could not be obtained for the remaining seven *Borrelia* spp. qPCR positive samples. All three genospecies were detected in woodland habitat, whereas woodland edge habitat had ticks infected with *B. garinii* and *B. valaisiana* only, and only *B. garinii* was found in under canopy habitat.

The density of infected nymphs was low overall, at 0.03 per 100 m² (95% CI 0.02-0.06). Lack of infected nymphs detected in open and under canopy habitats meant DIN was zero in both. Although infected nymphs were detected in woodland edge, density was low, giving a DIN of 0.02 (95% CI 0.003-0.08) per 100 m². DIN in woodland habitat was 0.11 (95% CI 0.05-0.20) per 100 m². Of all the locations with infected nymphs, DIN only exceeded 1 per 100 m² at a single location which was an urban woodland habitat in Southampton (Supplementary).

Finally, the best-fit model for *Borrelia* spp. prevalence included tick life stage only. Adults had a significantly higher prevalence compared to nymphs (Table 4). City was not significant (χ^2 (2) = 3.35, p = 0.187), nor was habitat (χ^2 (3) = 1.58, p = 0.663) or season (χ^2 (2) = 0.05, p = 0.973). Effects of

green space connectivity were also not found to be significant on *Borrelia* spp. prevalence. Distance to woodland (χ^2 (1) = 0.05, p = 0.819) and the proportion of green space at any buffer distance (250 m; χ^2 (1) = 0.02, p = 0.865, 1 km; χ^2 (1) = 0.23, p = 0.613) were not significant.

Discussion

Our study shows that urban green spaces can support *I. ricinus* ticks infected with *Borrelia* spp. in southern England. Although overall nymph density, *Borrelia* spp. prevalence and DIN was low, reports of millions of visits to urban green spaces annually (Bristol City Council, 2015; Southampton City Council, 2008), could mean there is a risk to public health due to potential transmission of Lyme borreliosis. Risk could be elevated in urban areas if tick awareness is low (Bayles et al., 2013). Tick presence and density varied significantly between cities, with Bristol, the largest and least connected city having consistently lower presence and density compared to Bath and Southampton. This finding is consistent with the latest Lyme borreliosis incidence rates published by the UK Health Security Agency, where incidence was lowest in Bristol (3.6/100,000) compared to Bath (5.1/100,000) and Southampton (4.4/100,000) (during 2021; (UK Health Security Agency, 2022). Woodland within cities were key habitats for both presence, density and *Borrelia* spp. prevalence and their importance increased during spring and summer when the highest presence and densities were found. This key finding in our study is similar to other studies in urban green space, where densities of *I. ricinus* have been highest during spring and summer (Buczek et al., 2014; Dobson et al., 2011; Hansford et al., 2017; Kowalec et al., 2017; Marchant et al., 2017). Although woodland edge has been highlighted as an important green space habitat for nymph density in previous peri-urban studies (Hansford et al., 2017; Mathews-Martin et al., 2020), woodland habitats away from edges had significantly higher densities of nymphs in this study and are likely key locations for humans to potentially encounter ticks within an urban landscape (Chvostáč et al., 2018; Hansford et al., 2021; Marchant et al., 2017; Vourc'h et al., 2016). Perhaps the reduction in suitability of

woodland edge within Bath, Bristol and Southampton was due to the central locations sampled and elevated temperatures reported in cities. Although trees can significantly reduce urban temperatures (Bowler et al., 2010), perhaps the beneficial effects of urban tree canopies or woodland for ticks (Gray, 2002) are not fully experienced at the edges of these important habitats in urban spaces. This would be exacerbated if woodland edges in cities are more abrupt (narrow, without a gradual change in vegetation composition) compared to potentially wider ecotones in peri-urban or rural woodland edge. Alternatively, host usage of edge habitat may be different in cities compared to the surrounding landscape, because of greater human disruption and other host barriers (e.g. roads) that may influence host behaviour (Passoni et al., 2021).

For the first time in England, connectivity between urban green space patches was shown to be a key driver of tick presence and density within urban green space, with similar findings to those reported in Antwerp, Belgium by Heylen et al., (2019). Reduced distance to woodland increased tick presence and density, particularly during spring and summer. This supports findings by Heylen et al., (2019), where higher densities of nymphs were associated with better connectivity. The importance of woodland habitat and its proximity is likely driven by woodland suitability for key tick hosts, including deer, small mammals, and birds (Estrada-Peña, 2002). All of these hosts can benefit significantly from woodland habitat (Evans et al., 2009; Lovari et al., 2017; Perez et al., 2016), and can provide a blood meal for ticks residing in or introduced into urban green space (Heylen et al., 2019; Maaz et al., 2018; Majerová et al., 2020). Roe deer are one of the most abundant and widespread ungulate species across Europe, with an ecological plasticity that enables adaptation to a wide range of habitats, including human dominated landscapes (Wevers et al., 2020). It is also one of the most abundant and widespread deer species in the UK (The British Deer Society, 2021) and a key tick host, feeding all stages of *I. ricinus* (Gray et al., 2021; Gray and Ogden, 2021). Although the link between landscape topography or heterogeneity is not always a significant predictor of roe deer home ranges (Morellet et al., 2013), the density of *I. ricinus* in urban green space has been shown to

increase with the degree of connectivity to known locations of deer populations (Heylen et al., 2019).

Ticks were collected from a wide range of locations across the three cities, but with stricter criteria for survey locations to fall fully within urban boundaries, overall presence of ticks was much reduced compared to a similar study in and around the city of Antwerp, Belgium (80%; Heylen et al., 2019). Overall nymph density in our study was much lower (1.22 per 100 m²) compared to a recent European estimated average (12.2 per 100 m²; Hansford et al., 2022) and other urban and peri-urban studies conducted in England where overall densities ranged from 2.6-26.1 per 100 m² (Greenfield, 2011; Hansford et al., 2021, 2017; Nelson et al., 2015). This is likely due to the inclusion of both suitable and less suitable urban patches falling within the city boundaries of Bristol, Bath and Southampton and highlights the importance of clarifying what is considered urban green space, so that assessments of risk in urban areas can be accurately formulated.

Although tick presence and nymph density were significantly lower in under canopy habitat, these spaces could also be important from a human tick exposure perspective, particularly if people use these spaces to seek shade or shelter when using urban green spaces. As reported in other urban studies (Hansford et al., 2021; Heylen et al., 2019; Krčmar et al., 2014; Maetzel et al., 2005; Nelson et al., 2015; Oechslin et al., 2017), ticks were virtually absent from open, managed habitat which mostly had short vegetation. Low vegetation is known to be unsuitable for ticks because it lacks the microclimate required to support and sustain water balance in ticks (Hansford et al., 2017; Herrmann and Gern, 2015). Although not specifically tested in this study, this is likely significant from a management perspective because it provides further data to the growing body of evidence that tick risk could potentially be reduced with grass cutting (Dobson et al., 2011; Hansford et al., 2021).

Borrelia spp. prevalence in nymphs was 2.8%, which is low compared to a recent European estimated average of *I. ricinus* in urban green space (Hansford et al., 2022), but similar to a wider

rural habitat study in the UK where on average, 3.8% of nymphs are infected with *Borrelia* spp. (Cull et al., 2021). Adult ticks consistently had higher infection rates compared to nymphs, which is widely reported elsewhere in Europe (Hubálek and Halouzka, 1998; Rauter and Hartung, 2005; Strnad et al., 2017), and was likely due to the additional blood meal taken by adults that increases the chances of infection (Braks et al., 2016).

Most positive ticks were infected with *B. valaisiana*, which is often the second-most common *Borrelia* genospecies reported in England (Cull et al., 2021), and the pathogenicity of which is still uncertain (Kiewra and Lonc, 2012). *Borrelia garinii* was also detected, and both are associated with transmission between ticks and birds (Dubska et al., 2009; Majerová et al., 2020). Birds are key tick hosts, providing blood meals for juvenile tick life stages, and many ground feeding species play a key role in the transmission of *Borrelia* spp. During feeding, ticks can be transported on birds over large distances, including into peri-urban and urban areas, where they likely play a significant role as a *Borrelia* spp. reservoir. Ground feeding birds (e.g., blackbirds or song thrushes, the latter of which are less common) are most likely to encounter questing ticks. These key hosts contribute to the maintenance of tick populations and *Borrelia* spp. transmission cycles, specifically bird associated *B. garinii* and *B. valaisiana* (Dubska et al., 2009; Majerová et al., 2020).

Blackbirds (*Turdus merula*), having historically been associated with forest habitat, are now one of the most adapted urban species across Europe, moving from nearby rural areas to towns and cities where they are found in higher densities compared to rural habitats (Evans et al., 2009). They are also an important host for feeding and infecting *I. ricinus* with *Borrelia* spp. (Gryczyńska and Kowalec, 2019). Whether blackbirds are introducing ticks from surrounding rural areas, or simply feeding ticks that are transported into urban areas by other wildlife remains to be determined, but the dominant genospecies found in this study suggests likely involvement of blackbirds in urban *Borrelia* spp. transmission cycles. Alternatively, grey squirrels (*Sciurus carolinensis*) could be involved in the transmission of *Borrelia* spp. genospecies in urban or peri-urban areas in southern England,

having been found infested with *I. ricinus* and infected with several genospecies commonly found in the UK (Craine et al., 1995; Millins et al., 2015).

Overall DIN reported in this study was lower compared to a recent European estimated average (1.7 infected nymphs per 100 m²; Hansford et al., 2022). Although overall DIN in Bath, Bristol and Southampton is very low compared to some urban studies which have reported between 5-10 infected nymphs per 100 m² (Marchant et al., 2017; Vourc'h et al., 2016), this may reflect the exclusion of any surrounding peri-urban or rural habitat and the wide range of habitat types sampled (which included both suitable and less suitable patches). We suggest that low DIN does not necessarily mean low risk, particularly with such high numbers of visitors using the urban green spaces in the cities studied.

Whilst the landscape metrics used were similar to another study (Heylen et al., 2019), they are still relatively simplistic indicators of connectivity and landscape scale ecological processes that might impact tick/host distribution. Incorporating additional metrics such as patch size and isolation, as well as datasets on the movement of people across urban landscapes would better identify high risk urban spaces. Excluding some green space types may have missed key tick areas such as cemeteries (Hornok et al., 2014). Additionally, some smaller urban patches or woodlands may have been missed, if they were too small to be included in Urban Atlas (2012) or Forestry Commission data (which only includes woodland over 0.5 ha). Improved seasonality assessments could have been obtained with more regular sampling (weekly), but this was not possible due to time constraints. Despite this shortfall, this study was still able to identify broad trends in seasonal differences, which are in line with other published results (Buczek et al., 2014; Hansford et al., 2017; Heylen et al., 2019; Kowalec et al., 2017; Marchant et al., 2017; Oechslin et al., 2017). Although the risk of tick-borne diseases can be estimated using various metrics such as nymph density, pathogen prevalence or the density of infected nymphs (Estrada-Peña and De La Fuente, 2014; Gilbert, 2021; Kilpatrick et al., 2017), the lack of collection of tick-bite data, Lyme borreliosis case data or human usage of urban

patches during this study limits these results. Understanding how members of the public move through urban green space, their activities within them, and any subsequent tick bites or Lyme borreliosis cases could enhance current estimates of risk. Finally, although risk factors within cities appear to be similar e.g., presence of woodland will likely increase the risk of contact between ticks and members of the public, the overall risk of one city compared to another may differ. It would be interesting, but logistically challenging, to expand this research to cover a larger number of cities.

This multi-city study confirms the presence of *Borrelia* spp. infected *I. ricinus* in a range of urban green spaces and highlights woodland habitat as a key risk area. Reduced distance to woodland was positively associated with tick presence and nymph density across all three cities and could be further investigated to potentially identify key risk areas in other cities, although unique city landscapes may reduce the sensitivity of this metric. This study establishes where and when ticks might be found in urban green space and investigated several connectivity factors possibly driving this, but it does not address human-tick contact rates, how many tick-bites occur or how many of these develop into Lyme borreliosis. This is a key future question for urban tick research..

Author statement

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Figure 1 OpenStreetMap of road networks and green spaces in the City of Bath with urban boundary and numbered sites randomly selected for sampling. Grey line is the Office for National Statistics Major Towns and Cities (2015) boundary. Contains OS data © Crown copyright and database right (2017).

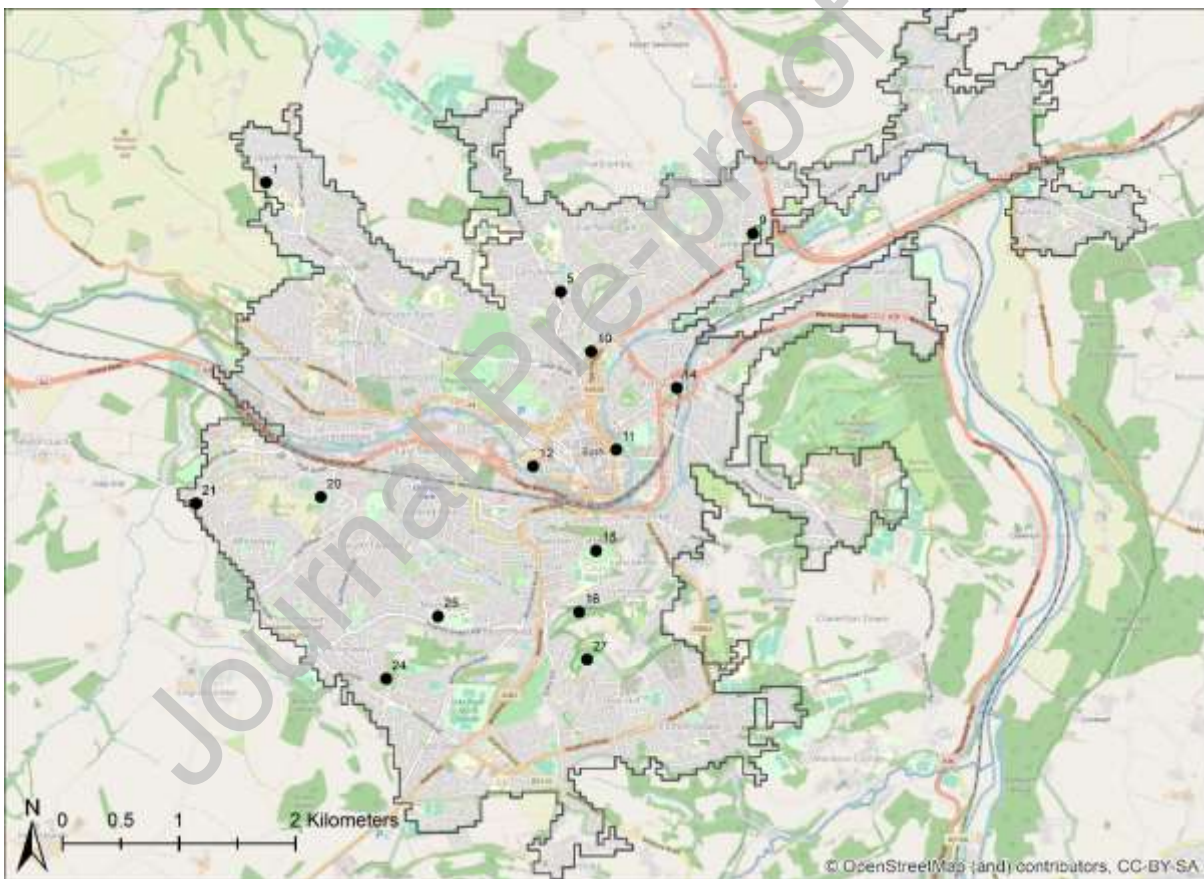


Figure 2 - Tick presence (one or more of any life stage) per habitat in the cities of Bath, Bristol, and Southampton during all seasons in 2017. Error bars represent 95% confidence intervals.

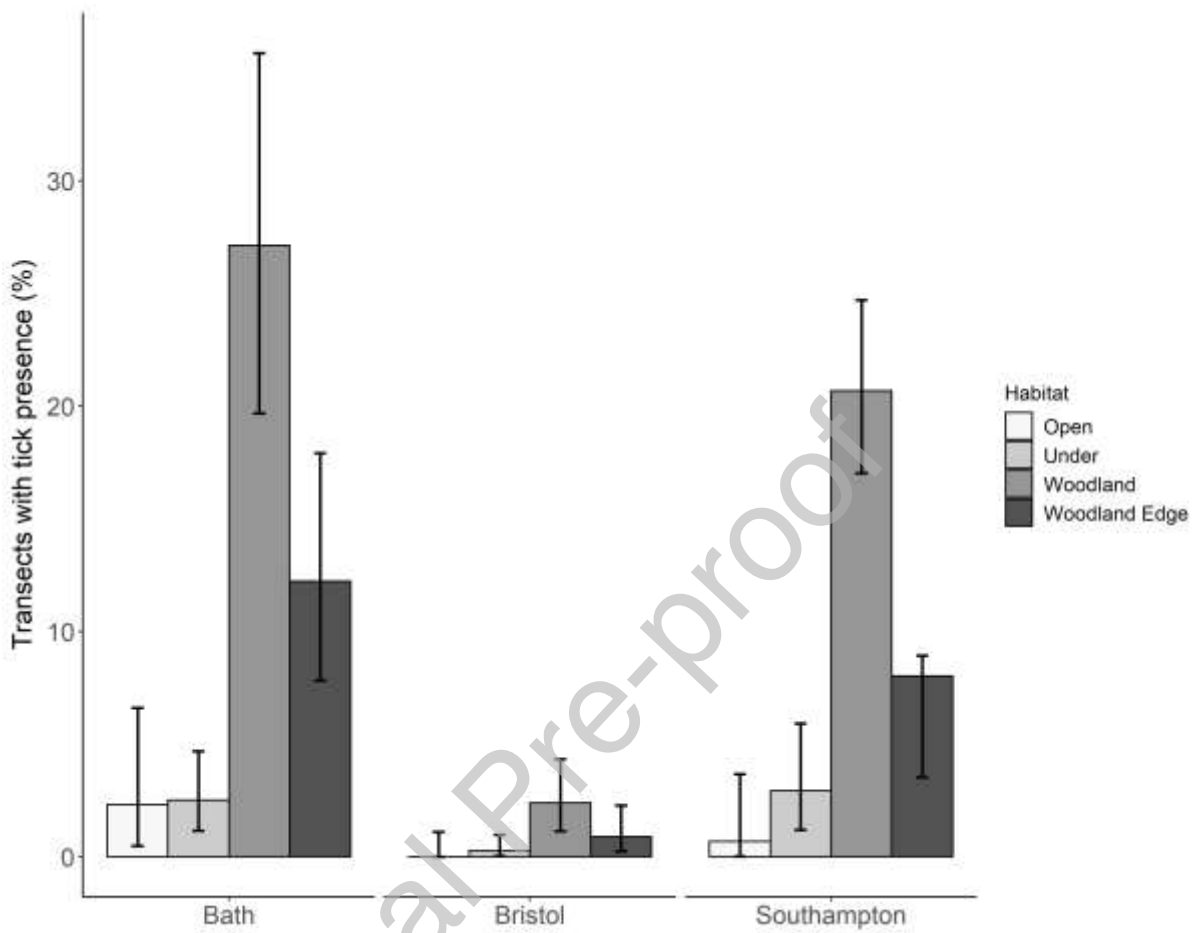


Figure 3 - Predicted presence of *Ixodes ricinus* in urban green space in relation to distance to woodland habitat. Shaded area represents 95% confidence intervals.

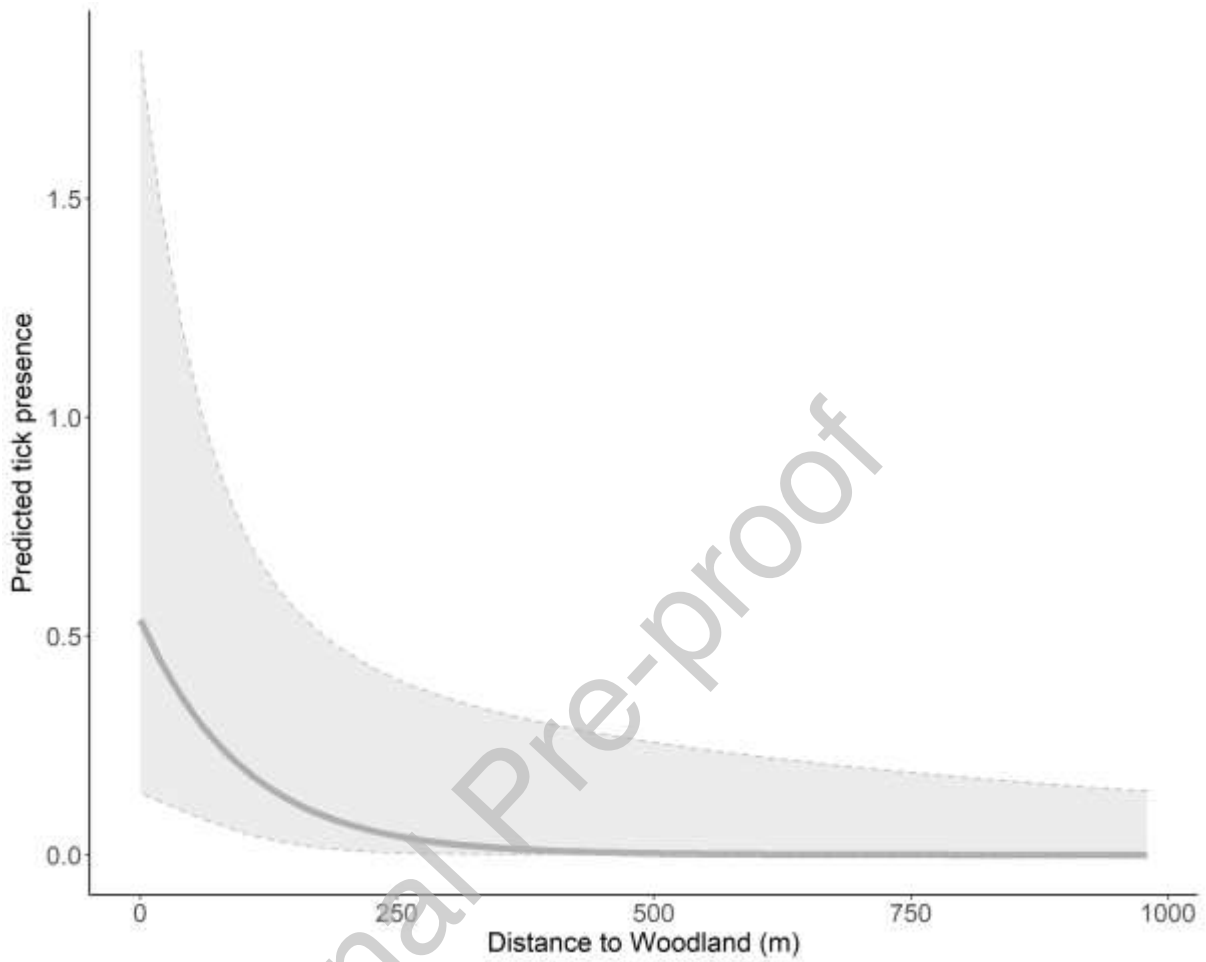


Figure 4 - Predicted density of *Ixodes ricinus* nymphs in relation to the proportion (%) of green space within 250 m of a sampling location, separated by habitat. Shaded areas represent 95% confidence intervals.

