



Recreational hazard: Vegetation and host habitat use correlate with changes in tick-borne disease hazard at infrastructure within forest stands

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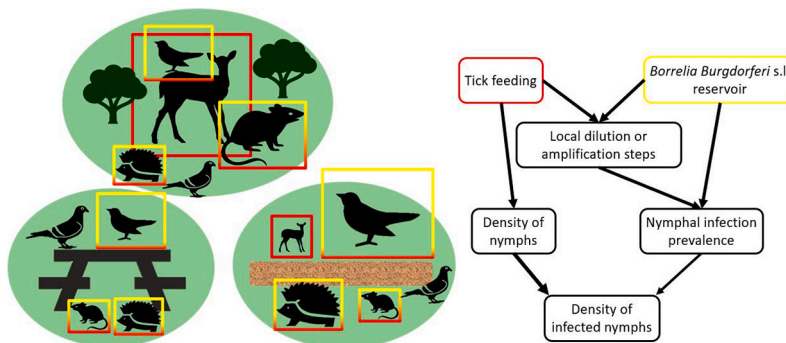
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HIGHLIGHTS

- Understanding micro-geographic variation in Lyme disease hazard can reduce risk.
- Hazard is low at forest edges and infrastructure, but high at interior forest trails.
- This is attributed to differences in vegetation and their effect on host abundance.
- Vegetation and host abundance drive both nymph density and infection prevalence.
- Tick density drives local hazard, but effects of pathogen prevalence are measurable.

GRAPHICAL ABSTRACT



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ABSTRACT

Studies on density and pathogen prevalence of *Ixodes ricinus* indicate that vegetation and local host community drive much of their variation between green spaces. Contrarily, micro-geographic variation is understudied, although its understanding could reduce disease risk. We studied the density of infectious nymphal *Ixodes* sp. ("DIN", proxy for disease hazard), density of questing nymphs ("DON") and nymphal infection prevalence ("NIP") near recreational forest infrastructure. Drag sampling within forest stands and at adjacent benches and trails was combined with vegetation surveys, camera trapping hosts and pathogen screening of ticks. We analysed *Borrelia burgdorferi* s.l. and its genospecies, with complementary analyses on *Rickettsia* sp., *Anaplasma phagocytophilum*, *Neohhrlichia mikurensis* and *Borrelia miyamotoi*. DIN was highest in forest interior and at trails enclosed by forest. Lower disease hazard was observed at benches and trails at forest edges. This infrastructure effect can be attributed to variation in vegetation characteristics and the habitat use of tick hosts, specifically roe deer, rodents and songbirds. DON is the main driver of DIN at micro-geographic scale and negatively affected by infrastructure and forest edges. A positive association with vegetation cover in understorey and canopy was observed, as were positive trends for local rodent and songbird abundance. NIP of different pathogens was affected by different drivers. Lower *B. burgdorferi* s.l. prevalence in the interior of forest stands, driven by its most

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prevalent genospecies *B. afzelii*, points towards higher density of uninfected hosts there. *B. afzelii* was positively associated with understorey containing tall species and with high canopy cover, whereas local bird community composition predicts *B. garinii* prevalence. A positive effect of songbird abundance and a negative effect of pigeons were observed. Our findings support amplification and inhibition mechanisms within forest stands and highlight that the effect of established drivers of DIN may differ based on the considered spatial scale.

1. Introduction

Public green spaces provide a multitude of ecosystem services (Brockhoff et al., 2017). Many of these services are linked to recreational use by visitors (Bolund and Hunhammar, 1999; Morse et al., 2022). Within green spaces, these visitors may be exposed to vectors of zoonotic disease, such as mosquitos and ticks. In Europe, tick-borne diseases (TBDs), which are predominantly transmitted by ticks of the *Ixodes ricinus* species complex, are more common than mosquito-borne diseases. The most prevalent TBD is Lyme borreliosis, which is caused by spirochetes of the *Borrelia burgdorferi* s.l. complex. Lyme borreliosis has been on the rise, followed by a stagnation in its incidence in recent decades, leading to its classification as a “high priority disease group” in Belgium (Burn et al., 2023; Hofhuis et al., 2015; Klammer et al., 2021; Mead, 2015, sciensano.be). This increase in observed incidence is likely owed to both an increase in actual incidence as well as increased testing, improved diagnostic techniques and surveillance. The risk of a specific TBD can be estimated as the contact rate between humans and infected ticks that are searching for a host by “questing”: vertical movement up vegetation combined with a sit-and-wait strategy. Given that most infections in humans are established by the nymphal life stage of ticks, this contact rate in turn depends on human behaviour and the density of infected nymphs (“DIN”) for this pathogen (Braks et al., 2016). The latter is universally accepted as a measure for TBD hazard and is a product of the density of questing nymphs (“DON”) and the infection prevalence in nymphs (nymphal infection prevalence, “NIP”) (Perret et al., 2004).

Two main drivers of both tick density and pathogen prevalence have been identified in the literature: (1) microclimatic variation and (2) the presence and abundance of different host species (Ehrmann et al., 2017; Medlock et al., 2013). Microclimate is characterised by fine scale variations in temperature and humidity which are decoupled from the background atmosphere by topography, landscape composition and vegetation (Bramer et al., 2018). Tree canopy is the main factor decoupling the local microclimate from macroclimate in forested areas (Dobrowski, 2011; Geiger et al., 2009; Kraus, 1911; Landuyt et al., 2019). In the understorey, vegetation creates both a suitable questing substrate and microrefugia suitable for long term tick survival (Daniel, 1993; Lees, 1946; Mejlon and Jaenson, 1997). Low temperature and relative humidity in the understorey diminish survival, increase interstadial development time and reduce questing activity, all of which reduces population growth (Brunner et al., 2012; Dautel et al., 2016; Gern et al., 2008; Gray et al., 2021; Jaenson and Lindgren, 2011). High temperature (> 30 °C) has a similar effect on survival and questing activity, as desiccation is stimulated by an increase in vapour pressure deficit (Lees, 1946). The effects of cold temperature and high vapour pressure deficits on questing activity reduce the contact rates with possible hosts, reducing the proportion of ticks that manage to complete their life cycle (Knülle and Rudolph, 1982; Sonenshine, 1991).

A second driver of DIN that has been identified is the host community, as both its composition and the abundance of vertebrate species have been shown to effect DON and NIP. Large vertebrates such as ungulates are needed to establish viable tick populations (Gilbert et al., 2012; Gray, 1998; Hofmeester et al., 2017b). They act as propagation hosts for *I. ricinus*, hosting the majority of reproductive stages (i.e. adult female and male). The density of ticks (and therefore DON) is further mediated by the availability of smaller vertebrate hosts on which the immature developmental stages (i.e. larvae and nymphs) feed

(Randolph, 1998).

Most zoonotic pathogenic agents transmitted by *I. ricinus* are not transmitted vertically (i.e. from mother to larva). Rather, pathogens like *Borrelia burgdorferi* s.l propagate in certain taxa of ticks hosts (the “reservoir” of the pathogen) and ticks are infected by taking a bloodmeal from an infected host. This is why the contact rate between larvae and competent reservoir hosts is expected to drive not only the density of nymphs, but also the nymphal infection prevalence in ticks (Hofmeester et al., 2016; Krawczyk et al., 2020). Due to the lack of vertical transmission, nymphs carrying taxon-specific pathogens can be assumed to have acquired them from their larval host (Gern and Rais, 1996; Humair et al., 1999). Given the very limited dispersion potential of larval and nymphal ticks (Gray, 1985; Milne, 1950a, b), it is to be expected that nymphal infection rates reflect the local abundance and habitat use of reservoir hosts. One example of a pathogen species that is exclusively transmitted horizontally is *Anaplasma phagocytophilum*, which can establish flu-like symptoms in infected humans (Rikihisa, 2011). The species *A. phagocytophilum* consists of four ecotypes, which have been associated with various vertebrate species, although the main reservoir of the zoonotic ecotype consists of ungulates and small mammals (Fabri et al., 2022; Jahfari et al., 2014; Stuenkel et al., 2013). The closely related *Neoehrlichia mikurensis* can establish asymptomatic or inflammatory infections when it is transmitted from its rodent reservoir to humans by *I. ricinus* (Burri et al., 2014; Portillo et al., 2018). In the case of *Borrelia burgdorferi* s.l., the causative agent of Lyme disease, the reservoir has been shown to consist of a wide variety of taxa. However, *B. burgdorferi* s.l. consists of a number of genospecies which differ in their pathogenicity for humans, as well as in their vertebrate reservoir hosts (Kurtenbach et al., 2006). In the terrestrial system of North-Western Europe, rodents and small mammals are the main reservoir for *Borrelia afzelii* (Hanincová et al., 2003a), while songbirds are the reservoir for *B. garinii*, *B. valaisiana* and *B. turdi* (Hanincová et al., 2003b; Heylen et al., 2016; Heylen et al., 2013). *B. afzelii* and *B. garinii* are among the most prevalent agents causing Lyme disease (Piesman and Gern, 2004; Rizzoli et al., 2011), with the latter associated with neuroborreliosis (Stanek and Reiter, 2011).

In the case of vertically transmitted pathogens, prevalence may not be directly related to local abundance of larval hosts, but rather to the population dynamics of the ticks themselves, as they (partly) act as reservoir (Sprong et al., 2009). Suitable microclimate and host availability determine the chances of ticks surviving to reproduction (see above), but the pathogen status of ticks may have an effect on survival and/or questing behaviour (Benelli, 2020; Herrmann and Gern, 2010). Despite the role of survival in the maintenance of vertically transmissible pathogens, it is assumed that their prevalence is still mediated by the availability of their respective reservoir species. For *Rickettsia helvetica*, a member of the spotted fever group rickettsiae, songbirds have been shown to be a potential reservoir (Heylen et al., 2016), whereas *Borrelia miyamotoi* circulates between rodents via ticks (Burri et al., 2014; Cosson et al., 2014).

Despite the aforementioned knowledge of the drivers of TBD hazard at large spatial scales (Ehrmann et al., 2017; Estrada-Peña, 2001; Medlock et al., 2013; Tack et al., 2012b), its variation within and between forest plots of a single green space remain poorly understood (Dobson et al., 2011; Gray et al., 2021; Kiewra et al., 2017; Millins et al., 2017; Vourc'h et al., 2016). In this study we aim to better understand variation in DIN on a within-forest stand scale (a “stand” being defined as an area with continuous vegetation type, uninterrupted by trails or other

infrastructure). To study this variation, we focus on recreational infrastructure in forests and parks (henceforth: green spaces), such as walking trails and benches. We do so because we expect that the main drivers identified above (microclimate/vegetation and local host community/abundance) exhibit measurable variation near such infrastructure: both understorey and canopy layer vegetation is frequently maintained to fit management goals, creating clearings and altering microclimate through increasing direct sunlight radiation and airflow. The altered microclimate and heightened recreation pressure around infrastructure may subsequently influence the habitat use of the tick hosts. Recent publications investigating tick density near green space infrastructure endorse this assumption, as consistent variation in DON was observed (Mols et al., 2022; Verheyen et al., 2021). Similar to clearings created by infrastructure, the forest edge represents a sharp microclimate gradient (Honnay et al., 2005; Suggitt et al., 2011; Van-neste et al., 2020). This has been shown to affect the local abundance of different host species (Burri et al., 2014; Carrascal and Alonso, 2006; Tufto et al., 2020). We therefore expect a combination of infrastructure and edge effects on DIN to be at play in forest stands at green space edges. Furthermore, as green space visitors spend most of their time near recreational infrastructure, the study of TBD risk at such locations is highly relevant: approximations of hazard based solely on vegetation characteristics of nearby forest stands may be inaccurate (Verheyen et al., 2021).

In the light of the ticks' limited dispersion potential from the site of detachment, we formulate the following central hypothesis: variation in the components of TBD hazard (DON and NIP) within and between forest stands correlates with local abundance of larval hosts, which in turn is driven by recreation pressure and microhabitat (Fig. 1).

Within our aim to better understand the fine scale distribution of infected nymphs (DIN) and its components, DON and NIP, we place particular emphasis on differences between types of green space infrastructure such as trails and benches. We investigate (1) the variation in local abundance of potential tick hosts and vegetation characteristics near green space infrastructure and (2) whether the variation in these drivers correlates to variation in DON, NIP and DIN. We do so by

combining a drag sampling campaign with pathogen analyses, detailed vegetation surveys of drag transects and camera trapping of potential tick hosts, with emphasis on larval hosts. We specifically hypothesise within (1) that vegetation characteristics differ based on the type of infrastructure present, to a scale of 20 m. Subsequently, we expect measurable differences in local host abundance at the same spatial scale, driven by these differences in vegetation characteristics and either avoidance of or attraction to the recreational infrastructure. Given the role of hosts in the population dynamics of both ticks and their pathogens, we hypothesise within (2) that both DON and the NIP of the studied pathogens are driven by the observed variation in vegetation and host community characteristics. In order to quantify the proposed correlations in a causative network, we build piecewise structural equation models ("SEMs") (Lefcheck, 2016). Combining these SEMs with generalised linear mixed models focused on the specific hypotheses described above allows us to estimate direct effects of infrastructure and vegetation on the components of TBD hazard (DON/NIP/DIN) from indirect effects through alterations in the habitat use of the ticks' hosts.

2. Materials and methods

2.1. Study area

Throughout the present study, drag sampling was conducted in 10 green spaces located in the Campine region of Flanders, Belgium (Fig. 2). This region has a temperate climate with warm summers, with annual mean temperatures ranging from 10.7 °C to 12.2 °C and mean total precipitation from 650.2 mm/year to 1038.8 mm/year. The sampled green spaces are all publicly accessible for recreational purposes. Within these green spaces, 36 sampling plots were chosen (Fig. 2.A). Ticks were captured twice per year in each plot from 2018 to 2021, corresponding to the two peaks of tick activity established in literature (May-early July and August – October, respectively. Overview of sampling schedule: Fig. S.1.) (Cayol et al., 2017; Randolph et al., 2002). Within these plots, three sampling location types were defined based on the local recreational infrastructure: (1) the interior of the associated forest stand (an

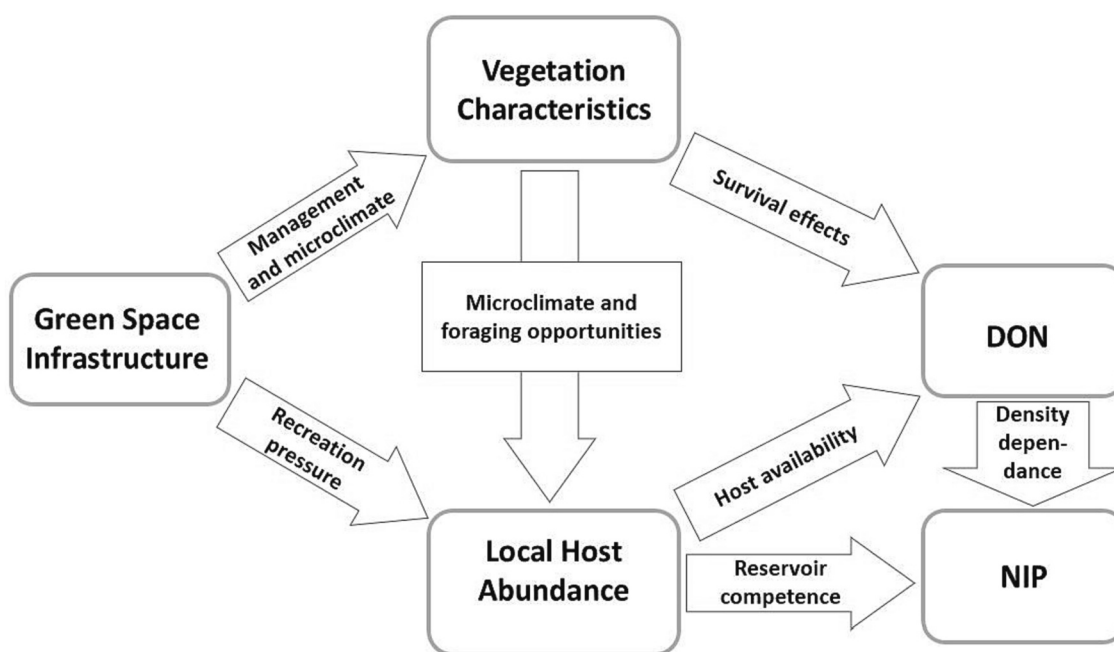


Fig. 1. Visualisation of the hypothetical pathways in the indirect effects of green space infrastructure on the density of questing nymphs (DON) and nymphal infection prevalence (NIP), through changes in vegetation characteristics, local host abundance and density dependence. The ecological processes supposedly driving the explanatory causal effects between variables are named in arrows.

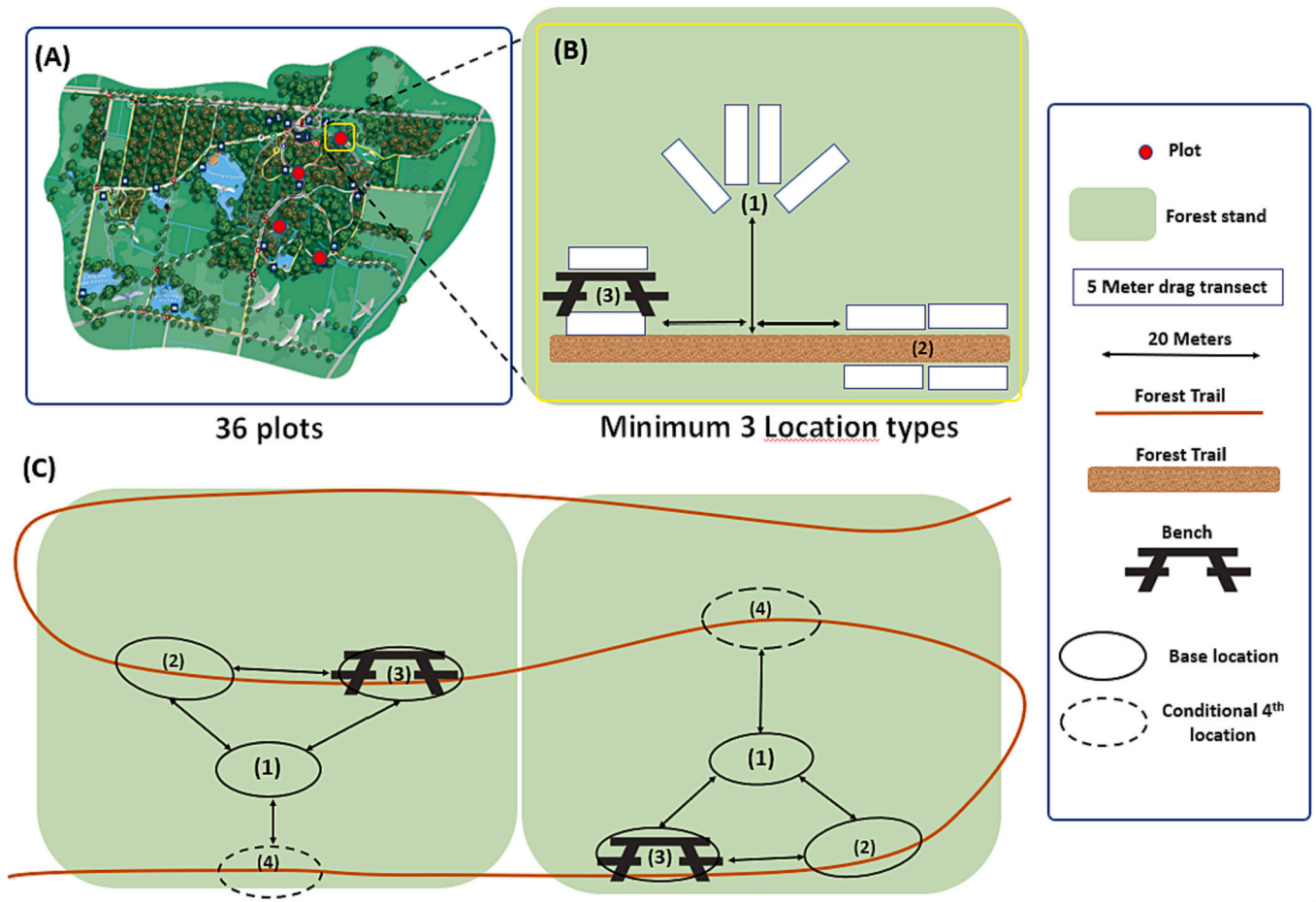


Fig. 2. Overview of the study setup with A) an example of a sample green space with four sampling plots; B) the core structure of each sampling plot, including (1) forest interior and recreational infrastructure (a (2)trail and (3) bench location) within a single forest stand; and C) the two possible configurations of plots consisting of four location types, with a bench at the forest interior, where the fourth location type is an edge trail (left) or with a bench at the forest edge in the core structure, where the fourth location type is an interior trail.

area with continuous vegetation type, uninterrupted by trails or other infrastructure. Henceforth “Forest interior”, examples in Fig. S.2); (2) an unpaved trail adjacent to the infrastructure (1–3 m wide, henceforth “trail”) and; (3) frequently used infrastructure (mostly benches, also picnic tables or information boards, henceforth “bench”). Furthermore, bench and trail locations were characterised as either in the forest interior (“interior bench”, “interior trail”), or at the forest edge (“edge bench”, “edge trail”), thus leading to five location types altogether. All plots consisted of at least a forest interior, bench and trail location (either interior or edge) (three locations per plot Fig. 2.B). The bench and trail locations were designated 40 m apart along the trail. Drag transects in the forest interior were selected to be representative for the vegetation in the forest interior, near a central point designated 20 m perpendicular to the trail, midway between the bench and trail. In 20 plots, we included an additional interior or edge trail location, connected with the former trail location through homogenous forest vegetation (4, Fig. 2.C). The fourth sampling location was only included whenever a suitable trail location was available at both the forest edge and interior (overview of plot composition: Table S.1). This setup is an extension of the sampling design described in Verheyen et al., 2021 where only three location types were included in each sampling plot.

2.2. Tick sampling

Collection of questing ticks was done by drag sampling, where 5 m² of litter and understorey vegetation were sampled with a white flannel

cloth of 1 m² (MacLeod, 1932). Where possible, we avoided dragging across bramble or woody debris, and wet substrate, as this may lead to sampling bias (Tack et al., 2011). At each sampling of a plot, four drag locations were selected as close to the centre of the sampling location in the interior of the forest stand (1) as possible, based on the suitability of the understorey. At location type (2), four drag samples were taken, two at either side of the trail. One drag sample was taken in front of the bench (3) (adjacent to the trail) and one behind. Wherever the infrastructural element was sufficiently large, one additional sample was taken at either side (resulting in four samples total per sampling instance). Ticks were identified to genus level based on morphological criteria upon capture and stored in 70 % ethanol at –20 °C until pathogen analysis. No determination using a microscope was performed, as we presume nearly all ticks captured by drag sampling in Flanders to be *I. ricinus*. Even so, all ticks captured in this way can be assumed part of the TBD hazard. Part of the gathered data on nymphal density (DON) were analysed and published in Verheyen et al., 2021.

2.3. Camera trapping and local host abundance

Camera traps were used to assess variation in local host abundance between plots and location types. Traps were placed at each location, secured to a seven-by-seven cm wooden pole, which was anchored in the soil with an iron foot. They were fixed at 40 cm above the soil, pointing slightly downward, at an angle of 10° to the pole. Browning strike force Pro X or Bushnell Trophy Cam models were used for sampling. During a

deployment, only one model type was used for all measurements within specific green spaces, to ensure comparability. Upon deployment, all non-woody vegetation higher than 10 cm was removed from the two by two meters sampling area in front of the setup, to standardise detection probability for each species between plots and location types. This setup is suitable to compare the local abundance of typically small larval hosts such as rodents and songbirds. It is however not suited to estimate absolute abundances.

The camera traps were programmed to record video files of 30 s, both during the day and at night. One sampling period lasted two weeks, after which video files were extracted and manually reviewed. If possible, recorded animals were determined to a species level, if not, to family level. For each sighting, the amount of time the animal spent within the sampling area was noted. If several individuals were visible at the same time, their individual times spent within the sampling plot were summed. This method does not take into account ecological or behavioural differences between species, as we do not attempt to estimate total abundance of species in a plot. Instead it focuses on the actual time each host species spends in suitable microhabitat for questing ticks (where drag sampling was conducted): a relative, local abundance as a proxy for the chance of fed larvae detaching there (hereafter “local (host) abundance”). Time spent by possibly infested hosts in other, nearby microhabitat is presumed irrelevant, due to the limited mobility of ticks. For further analyses, species were assigned to functional groups. Songbirds were defined as a functional group since they are the reservoir for avian *Borrelia* genospecies, notably *B. garinii* and *B. valaisiana* (Heylen et al., 2014). Pigeons were included in a separate group as their role as tick hosts, as well as their reservoir competence for pathogens is considered to be minimal (Fabbi et al., 1995). Rodents and shrews have been joined, as they exhibit similar tick loads and are reservoir species for (among others) *B. afzelii* (Gern et al., 1998; Jaenson et al., 2009; LoGiudice et al., 2003; Wolcott et al., 2021). Roe deer and muntjac were grouped (hereafter “Roe deer”, as only one observation of muntjac was made) because they are both tick propagation hosts and considered reservoir of the same pathogen species. This functional group was omitted from statistical analyses as sample size was low. Hedgehogs and squirrels, although similar in size and pathogen compatibility, were assigned to separate groups due to the considerable difference in their ecology.

2.4. Pathogen prevalences

Captured nymphs were screened for the presence of *B. burgdorferi* s.l., *R. helvetica*, *A. phagocytophilum*, *N. mikurensis* and *B. miyamotoi*. Alkine lysis was used to extract DNA (Schouls and Leo, 1999). Quantitative PCR (qPCR) was used to detect *B. burgdorferi* s.l. (Heylen et al., 2013), *B. miyamotoi* (Cochez, 2015), *N. mikurensis* (Jahfari et al., 2012) and *A. phagocytophilum* (Jahfari et al., 2014), as described in the respective references. *R. helvetica* was detected by duplex qPCR, aimed at *R. helvetica*, spotted fever and typhus group Rickettsia (Heylen et al., 2016; Stenos et al., 2005; PMID: 16354816). Sequencing of the variable 5S-23S (rrfA-rrlB) intergenic spacer (IGS) region was used to specify the genospecies of each *B. burgdorferi* s.l. positive sample (Coipan et al., 2013). Of samples positive for *A. phagocytophilum*, the groEL-gene was amplified and sequenced to determine the ecotype (Jahfari et al., 2014; Stuenkel et al., 2013).

2.5. Vegetation surveys

For every 5 m² drag transect, the percentage of substrate covered by living vegetation was estimated visually and categorised in intervals differing 5 % in value (5 %, 10 %, ... 95 %, 100 %, hereafter “total understorey cover”). The cover of individual species within these layers was noted with the same accuracy, and later aggregated in functional groups based on the expected magnitude of their effect on microclimate at soil level and supposed effect on host habitat use: (1) grasses, which have a limited effect on microclimate, given their low relative biomass;

(2) short herbaceous understorey species which are characterised by intermediate biomass and (3) tall understorey species with high biomass (including shrubs/bushes and tree saplings). We also estimated canopy cover and the proportion of this cover comprising of deciduous vegetation. For further analyses, the sum of these values was included (hereafter referred to as “total canopy cover”) as well as the proportion of deciduous and coniferous canopy.

2.6. Statistical analyses

statistical models were drafted in R to analyse variation in *Borrelia burgdorferi* s.l. hazard (DIN) and its components (DON and NIP) between drag samples. To estimate DIN, the DON of each drag transect was multiplied by the average NIP for the sampling instance of that specific location. For each response variable (DIN, DON and NIP) a base generalised linear mixed model (“GLMM”) containing location type and the interaction term between month and year (“Infrastructure model”) was expanded upon by adding either standardised local host abundance of all functional groups (with exception of roe deer, as sample size was low: “Host model”) or vegetation characteristics (the cover percentage of each functional group, standardised total understorey cover and standardised total canopy cover: “vegetation model”) as fixed explanatory variables (Fig. 3). Location type was included in all models as a fixed factor, nested in sampling plot which was in turn nested in green space (both random). Models for DON and DIN were drafted to the drag sample level ($n = 2377$, negative binomial error distribution), whereas models for NIP to the individually tested tick ($N = 6683$, using a binomial error distribution). This resulted in six GLMMs concerning *B. burgdorferi* s.l. hazard (vegetation and host model for DIN, DON and NIP, respectively). To estimate the effects of different reservoir species on the infection prevalence, vegetation and host models with the nymphal infection prevalence of the most prevalent genospecies of *Borrelia burgdorferi* s.l. (*Borrelia afzelii*, *Borrelia burgdorferi* s.s. and *Borrelia garinii*) were also drafted, resulting in six more models with binomial error distribution. The same was done for the nymphal infection prevalence and density of infected nymphs of the four other species of pathogen that were screened, resulting in eight more GLMMs with binomial error distribution (NIP) and eight with negative binomial error distribution (DIN).

Subsequently, a linear model (“LM”) was drafted to analyse the variation in each considered vegetation characteristic (including sampling month and the fixed factor for location type nested in the random factors sampling plot and green space). A similar LM was drafted with the local host abundance, expressed as the mean time each functional group spent at a given location. This averaging, combined with technical failure of camera traps and logistic difficulties resulted in 114 observations for each group. The average for each vegetation characteristic was included as fixed explanatory variable, as well as the location type (again nested within plot and, subsequently, green space).

In order to estimate the pathways through which green space infrastructure affects TBD risk, structural equation models (SEMs) were constructed (package: piecewiseSEM). All variables were averaged on the individual location level across the entire dataset, given that this is the sampling resolution of the limiting parameter (as mentioned above, local host abundance could not be calculated for all locations, final $N = 114$). Location type was included as an exclusively explanatory variable, whereas vegetation characteristics and local host abundances were used as both explanatory and dependent variables. The DON, as well as the NIP and DIN of *B. burgdorferi* s.l. were defined as strictly dependent variables based on the conceptual schemes in Fig. 1. The initial model for each dependent variable included all hypothesized pathways (“full model”). Alternative models were then drafted, only including effects corresponding to significant correlations in the (GLM(M)s described above for (“significance model”). A final model was built for DIN and its components by removing the direct effect of green space infrastructure on the ultimate dependent variable (DIN/DON/NIP). Models were

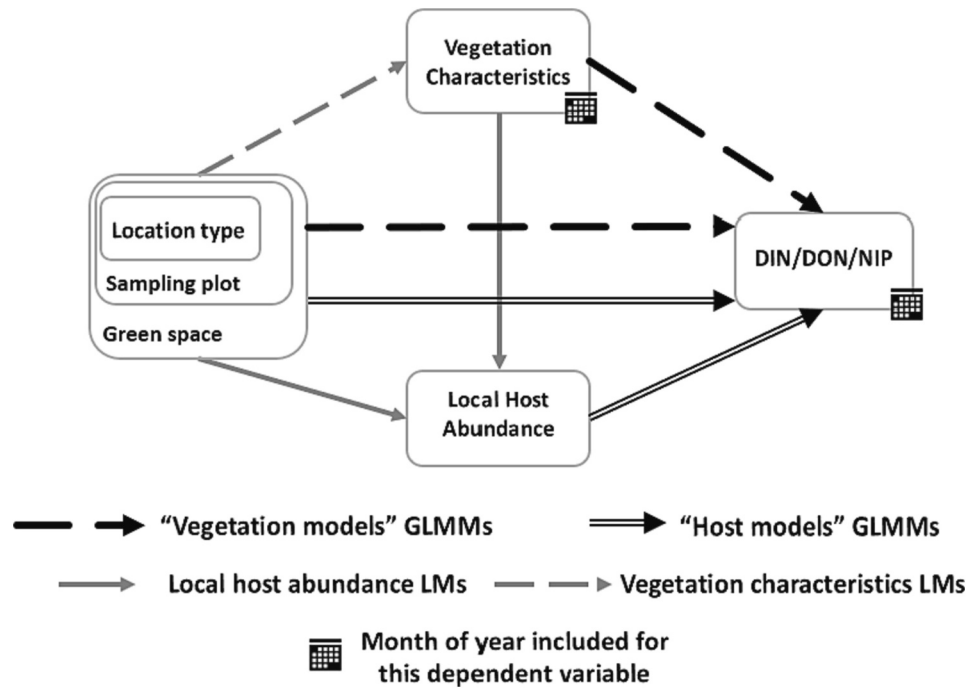


Fig. 3. visualisation of the models drafted during the statistical analyses within this study.

compared using the Akaike Information Criterion, corrected for small sample sizes (“AICc”) and individual pathways were estimated using the coefficients function.

3. Results

3.1. Lyme disease hazard and its components near recreational infrastructure

We measured an overall Lyme disease hazard, expressed in density of questing nymphs infected with *Borrelia burgdorferi* s.l. per m² (± standard error, henceforth “DIN”), of 0.10 ± 0.005. The most infected ticks per m² were observed in forest interior (0.13 ± 0.01) and at interior trails (0.13 ± 0.01). this was on average twice as high as the observed density of questing infected nymphs near benches, both at the forest

interior and edge (0.06 ± 0.01 and 0.07 ± 0.01, respectively). Trails at the forest edge were associated with the lowest DIN (0.04 ± 0.005). Lyme disease hazard was observed to be considerably higher in spring and summer (0.14 ± 0.01 and 0.13 ± 0.01, respectively) compared to autumn (0.03 ± 0.004). DIN was highest in the year 2020 (0.15 ± 0.02), followed by 2018 (0.10 ± 0.01), 2021 (0.09 ± 0.01) and 2019 (0.07 ± 0.01).

A total of 6890 nymphs were captured throughout the experiment, which translates to a mean total density of 0.60 ± 0.02 nymphs per m² (± standard error, henceforth “DON”). The nymphal density in forest interior was higher compared to that near infrastructural elements (0.88 ± 0.05). DON was second highest near interior trails (0.61 ± 0.04), followed by edge benches, interior benches and edge trails (0.40 ± 0.06, 0.29 ± 0.03 and 0.28 ± 0.02, respectively). Highest DONs were observed in spring (May–June: 0.90 ± 0.04), with intermediate overall

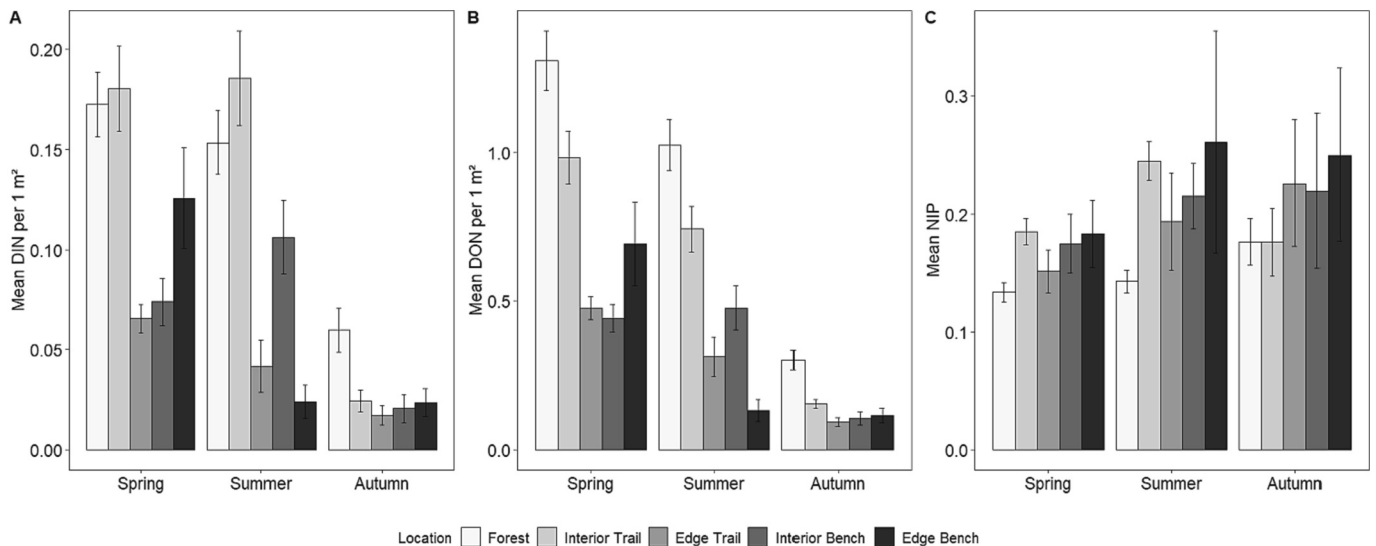


Fig. 4. Variation between location types and over time in (A) Lyme borreliosis hazard, expressed in Density of questing infected nymphs (mean ± se), and both its components: (B) Density of questing Nymphs and (C) the Nymphal infection prevalence of *Borrelia burgdorferi* s.l.

DON in summer (July – August: 0.70 ± 0.04) and lower values in autumn (September – October 0.18 ± 0.01 , Fig. 4, Fig. S.3). Among the four years of sampling, DON was highest in 2020 (0.75 ± 0.06), followed by 2018 (0.58 ± 0.03) and 2019 (0.58 ± 0.05). Mean DON was lowest in 2021 (0.43 ± 0.03).

Of the captured ticks, 6683 nymphs were screened for pathogens. The overall nymphal infection prevalence of *Borrelia burgdorferi* s.l. (fraction of nymphs infected \pm standard error, “NIP”) was 0.17 ± 0.009 , with highest values recorded at benches, both edge and interior, and interior trails (0.20 ± 0.03 , 0.20 ± 0.02 and 0.21 ± 0.01 respectively). NIP was significantly lower at edge trails (0.17 ± 0.02) and lowest in the forest interior (0.14 ± 0.01). The lowest infection prevalence was observed in spring (0.16 ± 0.01), whereas the proportion of captured nymphs that were infected with *B. burgdorferi* s.l. was higher in Summer (0.18 ± 0.01) and Autumn (0.19 ± 0.02). The highest percentage of nymphs was infected in 2021 (0.20 ± 0.01), followed by 2020 (0.19 ± 0.01), 2018 (0.17 ± 0.01) and 2019 (0.13 ± 0.01).

3.2. Density of infected nymphs: *Borrelia burgdorferi* s.l.

Within the GLMMs describing the DIN of *Borrelia burgdorferi* s.l., a positive correlation of DIN with canopy cover (0.20 ± 0.10 , $p = 0.05$) and a trend towards higher DIN at locations with a higher local songbird abundance (0.10 ± 0.05 , $p = 0.06$) were observed, in addition to a significant effect of location type (Table 1). The latter confirmed that a higher DIN persists in forest interior, although the difference with interior trails is insignificant.

3.3. Density of nymphs: vegetation characteristics and local host abundance

In both the vegetation and host models, the density of questing nymphs (DON) varied significantly between location types- (forest interior > interior trails > edge benches > interior benches > edge trails, as visualised in Fig. 4). Additionally, the vegetation model revealed a positive association between DON and both total understorey cover (0.11 ± 0.057 , $p = 0.05$) and the proportion of tall species in the understorey (0.80 ± 0.25 , $p < 0.001$). Also, higher nymph density was observed at locations with denser canopy cover (0.27 ± 0.06 , $p < 0.001$). The host model revealed no statistically significant correlations in addition to location type and the included time variable (interaction year:month). However, two trends were observed: DON exhibited a marginally positive correlation with local rodent abundance (0.11 ± 0.07 , $p = 0.06$) and was marginally higher at locations where songbirds spend more time (0.10 ± 0.06 , $p = 0.09$, Table 2).

3.4. Nymphal infection prevalence: *Borrelia burgdorferi* and its genospecies

The GLMMs exploring the nymphal infection prevalence (NIP) of *Borrelia burgdorferi* s.l. revealed a significant correlation with location type in both the host and vegetation models, with prevalence in the forest interior being lower than that near infrastructure ($p = 0.007$, Fig. 6, Table S.3). There were no significant additional associations with the abundance of hosts or vegetation characteristics ($p > 0.10$).

The most prevalent genospecies of *B. burgdorferi* s.l. that was taken into account was *B. afzelii* (9%), followed by *B. burgdorferi* s.s. (1%) and *B. garinii* (0.7%). For the infection prevalence of *B. garinii*, no correlation was found with recreational infrastructure, neither with the included vegetation characteristics (Table S.3). We did, however, observe significant negative correlation with the local abundance of pigeons (-3.18 ± 3.72) and a trend towards higher prevalence when local abundance of songbirds was high (0.40 ± 0.13). For the infection prevalence of *Borrelia afzelii* we observed a significant correlation with recreational infrastructure ($p = 0.04$). No additional correlations or trends with host abundances were observed (all $p > 0.10$), but a

Table 1

Predictors of the density of questing nymphs infected with *Borrelia burgdorferi* s.l., as observed in negative binomial GLMMs including vegetation characteristics (left) and local host abundance (right) as explanatory variables. Significance is expressed in p-value (bold if $p < 0.05$) and the test statistic is the Likelihood Ratio Test, including degrees of freedom in subscript.

Fixed effects	Test statistic	Estimate \pm SE	p
<i>DIN B. burgdorferi</i> s.l.			
Spatial variable			
Location type	LRT ₄ = 20.64		< 0.001
Forest interior		(intercept) -2.42 \pm 0.28	
Trail			
Interior		-0.20 \pm 0.19	0.32
Edge		-1.14 \pm 0.28	< 0.001
Bench			
Interior		-0.56 \pm 0.28	0.05
Edge		-0.54 \pm 0.35	0.11
Vegetation characteristics			
Understorey cover	LRT ₁ = 1.15	0.09 \pm 0.09	0.28
Proportion grasses	LRT ₁ = 0.43	-0.24 \pm 0.41	0.51
Proportion herbs	LRT ₁ = 0.4144	0.26 \pm 0.41	0.52
Proportion tall herbs	LRT ₁ = 1.05	0.35 \pm 0.34	0.31
Total canopy cover	LRT ₁ = 3.90	0.20 \pm 0.10	0.05
Proportion deciduous	LRT ₁ = 0.57	0.32 \pm 0.40	0.45
Time variable			
Year*Month	LRT ₈ = 6.08		0.63
Year	LRT ₃ = 2.38		0.50
Month	LRT ₅ = 76.47		< 0.001
<i>DIN B. burgdorferi</i> s.l.			
Spatial variable			
Location type	LRT ₄ = 14.96		0.004
Forest Interior (Intercept)		-5.05 \pm 0.57	
Trail			
Interior		-0.05 \pm 0.21	0.80
Edge		-1.09 \pm 0.33	0.001
Bench			
Interior		-0.22 \pm 0.34	0.51
Edge		-0.16 \pm 0.50	0.74
Local host abundance			
Rodents	LRT ₁ = 0.48	0.06 \pm 0.10	0.49
Songbirds	LRT ₁ = 5.80	0.10 \pm 0.05	0.06
Pigeons	LRT ₁ = 0.35	-0.08 \pm 0.15	0.55
Hedgehogs	LRT ₁ = 0.16	-0.02 \pm 0.09	0.69
Squirrels	LRT ₁ = 1.08	-0.11 \pm 0.11	0.29
Time variable			
Year*Month	LRT ₈ = 8.21		0.41
Year	LRT ₃ = 7.27		0.06
Month	LRT ₅ = 54.33		< 0.001

Table 2

Predictors of DON as computed in the negative binomial GLMMs including location type, time variables and either vegetation characteristics (top) or local host abundance (bottom). Significance is expressed in p-value (bold if $p < 0.05$) and the test statistic is the Likelihood Ratio Test, including degrees of freedom in subscript.

Response: DON			
Fixed effects	Test statistic	Estimate \pm SE	p
Vegetation characteristics			
Understorey cover	LRT ₁ = 3.84	0.11 \pm 0.057	0.05
Proportion grasses	LRT ₁ = 2.67	-0.41 \pm 0.25	0.10
Proportion herbs	LRT ₁ = 0.41	0.26 \pm 0.41	0.52
Proportion tall herbs	LRT ₁ = 13.58	0.80 \pm 0.25	< 0.001
Total canopy cover	LRT ₁ = 18.89	0.27 \pm 0.06	< 0.001
Proportion deciduous	LRT ₁ = 0.15	-0.098 \pm 0.25	0.70
Canopy openness	LRT ₁ = 10.13	-0.015 \pm 0.004	0.001
Local host abundance			
Rodents	LRT ₁ = 3.64	0.11 \pm 0.067	0.06
Songbirds	LRT ₁ = 2.58	0.10 \pm 0.061	0.09
Pigeons	LRT ₁ = 0.59	-0.05 \pm 0.06	0.44
Hedgehogs	LRT ₁ = 0.79	-0.02 \pm 0.06	0.79
Squirrel	LRT ₁ = 0.73	-0.02 \pm 0.06	0.74

significant positive relationship with canopy cover (0.18 ± 0.09) and the proportion of tall understorey species in the herb layer (0.77 ± 0.30) were observed, as well as a lower NIP with an increase in the proportion of deciduous vegetation in the canopy (-0.66 ± 0.32). A trend towards positive correlation of *B. afzelii* NIP with the proportion of understorey covered by grass species was also observed (0.66 ± 0.32). Neither the host model nor vegetation model analysing the NIP of *B. burgdorferi* s.s.

revealed any significant correlation.

3.5. Disease hazard of other pathogens

For all tested pathogens, density of questing infected nymphs (DIN) was on average highest in the forest interior (Fig. 5). As was the case for *Borrelia burgdorferi* s.l., the difference in density of nymphs infected with

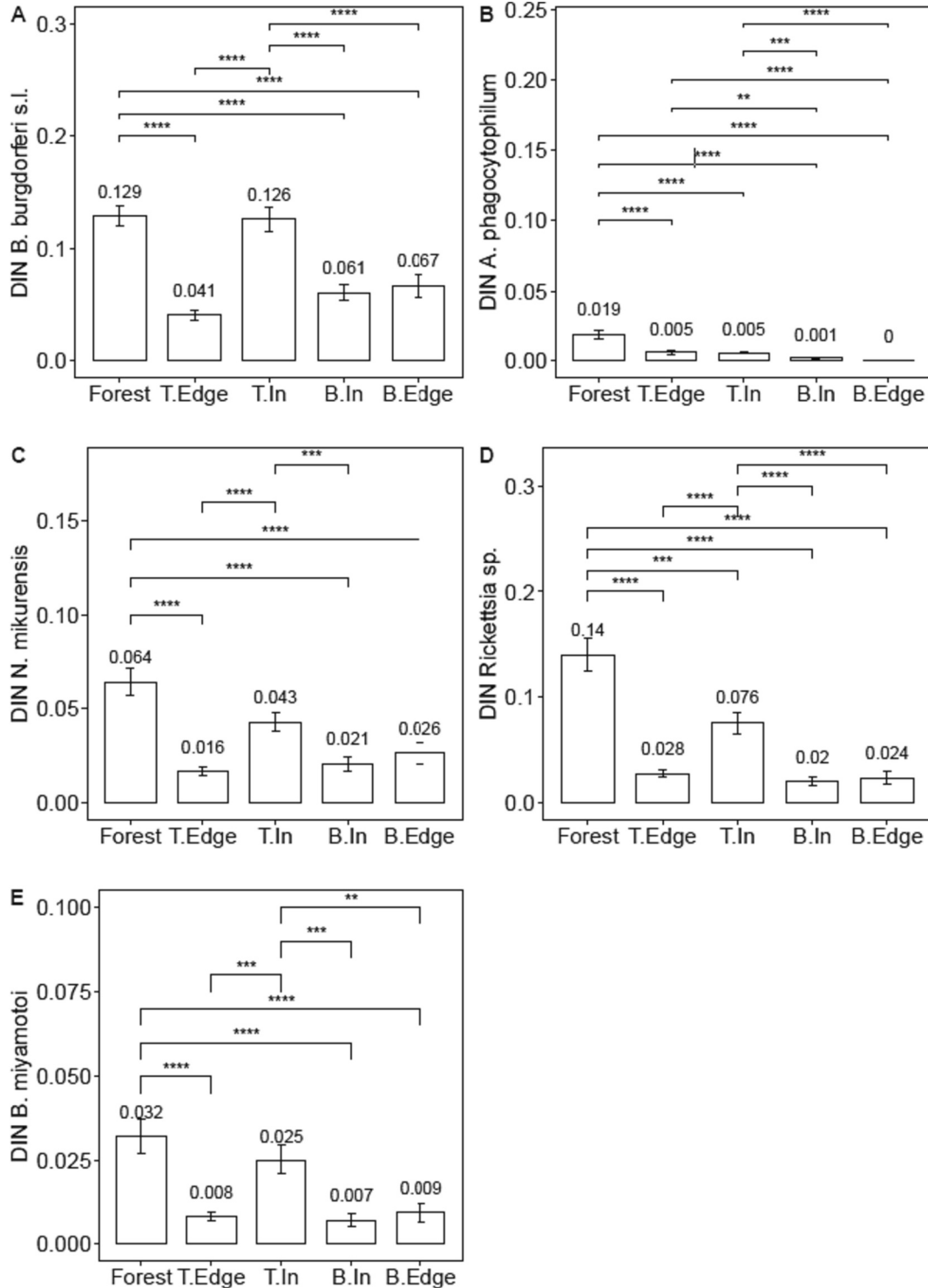


Fig. 5. Density of questing nymphs infected with screened pathogens (mean ± se infected nymphs per m²) for each location type (Forest = Forest stand interior, T.In = interior trail, T.Edge = edge trail, B.In = interior bench and B.Edge = edge bench). Pathogens include (A) *Borrelia burgdorferi* s.l.; (B) *Anaplasma phagocytophilum*; (C) *Neohrlichia mikurensis*; (D) *Rickettsia helvetica* and (E) *Borrelia miyamotoi* (Please note the differences in scale of the Y axes). The significance of the differences in DIN between location types are quantified as p-values of associated pairwise t-tests (ns p > 0.05, * p < 0.05, **p < 0.01, *** p < 0.001).

Borrelia miyamotoi between forest interior and interior trails was insignificant. There were no significant differences in disease hazard for any of the measured pathogens between different types of benches. The same was true for differences between edge trails and benches (either in the forest interior or at the forest edge), other than for *B. burgdorferi* s.l. (for which DIN was lowest at edge trails) and *Anaplasma phagocytophilum* (for which DIN was lowest at benches).

3.6. Nymphal infection prevalence of other pathogens

The causative agents of Lyme disease, *Borrelia burgdorferi* s.l., was the most prevalent pathogen detected in questing nymphs (17 % overall infection prevalence), followed by *Rickettsia* sp (13 %), *Neoehrlichia mikurensis* (7 %), *Borrelia miyamotoi* (4 %) and *Anaplasma phagocytophilum* (2 %). Of the 146 samples positive for *Anaplasma phagocytophilum*, 55 represent ecotype 1 (for which the main reservoir hosts are hedgehogs) and 47 ecotype 2 (for which ungulates are the reservoir species). The prevalence of *Rickettsia* sp. and *Anaplasma phagocytophilum* was highest in the forest interior (Fig. 6). For the latter, infection prevalence was similar at edge trails and forest interior.

The infection prevalence of *Anaplasma phagocytophilum* in nymphs correlated significantly with infrastructure type, in both the vegetation and host models ($p < 0.05$, Table S.3). NIP was also significantly lower when a high proportion of the canopy cover consisted of deciduous vegetation (-0.45 ± 0.69) and correlated marginally positively (0.41 ± 0.25) with the total canopy cover. No correlations with local host abundance were found for *A. phagocytophilum* prevalence (Table S.3). The NIP of *N. mikurensis* exhibited a significant, negative relationship with the local density of squirrels (-0.18 ± 0.10 ; Table S.3) and a trend towards a positive relationship with the proportion of herbaceous species in the herb layer (1.20 ± 0.66). No correlations were found between the NIPs of *B. miyamotoi* or *R. helvetica* with either location type, local host abundance or vegetation characteristics (Table S.3).

3.7. Contrasts in vegetation and host abundance between location types

3.7.1. Vegetation

The linear models exploring vegetation characteristics near recreational infrastructure revealed significant variation between location types for all response variables (Fig. S.4). Canopy openness was higher at edge location types and interior benches, whereas the forest interior was characterised by high cover in both understorey and canopy. The percentage of total herb layer cover consisting of short herbaceous species was highest at benches, whereas tall understorey species were most prevalent at interior trails.

3.7.2. Local host abundance

In total, 3560 recordings were made of potential tick hosts. Local abundance of rodents was higher in the forest interior, whereas hedgehog and songbird abundance was on average highest at trails (Fig. S.5). Pigeon abundance was highest at benches and that of squirrels at forest interior and interior trails. Roe deer were only observed in forest interior and a single time at an edge trail.

The linear models showed that local rodent abundance was significantly higher at forest interior, compared to locations at green space infrastructure ($p < 0.001$, Table S.2). Besides significantly ($p = 0.006$) higher local abundance at locations with lower canopy openness, trends ($0.5 < p < 0.1$) were observed for higher presence per day at locations with more canopy cover and a higher proportion of deciduous vegetation in the canopy layer. The local abundance of songbirds did not vary significantly among location types or correlate with any of the explanatory variables in our models. The local abundance of pigeons did not differ significantly between location types or vegetation characteristics ($p > 0.10$), with exception of higher abundance at locations with a high proportion of low herbaceous plants in the understorey ($p < 0.001$). Hedgehog abundance did not significantly correlate with location type,

although the pairwise comparison with forest interior using the “summary” function in R pointed towards a higher local density at trails ($p = 0.05$ for trails in the interior of stands, $p = 0.12$ for trails at forest edges). Significant correlations of hedgehog abundance with proportionately more herbaceous understorey vegetation were observed, for both tall and short herbaceous species ($p = 0.02$ for both). Other than a trend ($p = 0.07$) for lower local squirrel abundance at edge trails, no (near) significant effects of location type or vegetation characteristics on their habitat use were observed (table S.2).

3.8. Structural equation models

The AICc of the structural equation models describing DON was lower, the fewer parameters were included. Differences between the full models and models including only significant correlations observed in the GLMMs above were considerably larger ($\Delta AICc =$ approximately 1000) compared to the differences between similar models with and without direct effect of green space infrastructure on DIN ($\Delta AICc = 23.16$, final model AICc = 3177, Fig. 7). Similar differences in AICc values for full and “significance” models, as well as between models with and without direct effects of location type on the ultimate dependent variable persisted for the SEMs analysing variation in DON and NIP of *B. burgdorferi* s.l. (figure S.6 and S.7, respectively). Significant pathways were observed in the significance model for DON excluding the direct effect of green space infrastructure on DON.

4. Discussion

4.1. Density of infected nymphs

The results obtained support our central hypotheses that the hazard of tick-borne disease (expressed in the density of questing infected nymphs: “DIN”), and its components, density of questing nymphs (“DON”) and nymphal infection prevalence (“NIP”), vary significantly on micro-geographic scale near green space infrastructure. We also confirmed that significant differences in vegetation characteristics and local host abundance persist between locations with different recreational infrastructure (“location types”). For a number of host groups, vegetation characteristics explained additional variation in local abundance, additive to the location type. Our analyses revealed that DON, NIP of a number of pathogens and as a result DIN correlate with both vegetation characteristics and the local abundance of hosts. Combining these observations, we present evidence for the effects of vegetation characteristics on DON and NIP to act, at least in part, indirectly through differences in local host abundance. As was the case for all pathogens, DIN for *Borrelia burgdorferi* s.l. was highest in the forest interior and adjacent to trails within the forest interior. Although measured DON was higher in the interior of the forest stands, the higher NIP of *B. burgdorferi* s.l. at trails led to a similar Lyme disease hazard between the two location types. The lower DON (and therefore DIN) at trails at forest edges implies an edge effect through small-scale variation in vegetation characteristics and local host abundance. In addition, the lower DON/DIN at benches suggests an infrastructure effect which may result from the creation of small edge microhabitats and avoidance behaviour by hosts. These suggestions are reinforced by the correlations of DIN and NIP of *B. burgdorferi* s.l. with vegetation characteristics and the local density of larval hosts, although differences between genospecies were observed.

Models analysing variation in DIN for *B. burgdorferi* s.l. all included a significant factor for the month of year. Herein, DIN was highest in spring and summer, and decreased towards autumn. This is in line with the yearly patterns in questing activity described in the literature, endorsing the consensus that DON is the main driver of variation in DIN, rather than NIP, as the latter exhibits a smaller magnitude of variation (Hartemink et al., 2021; Hartemink et al., 2019; Ruyts et al., 2016). However, the similar Lyme disease hazard at forest interior and interior

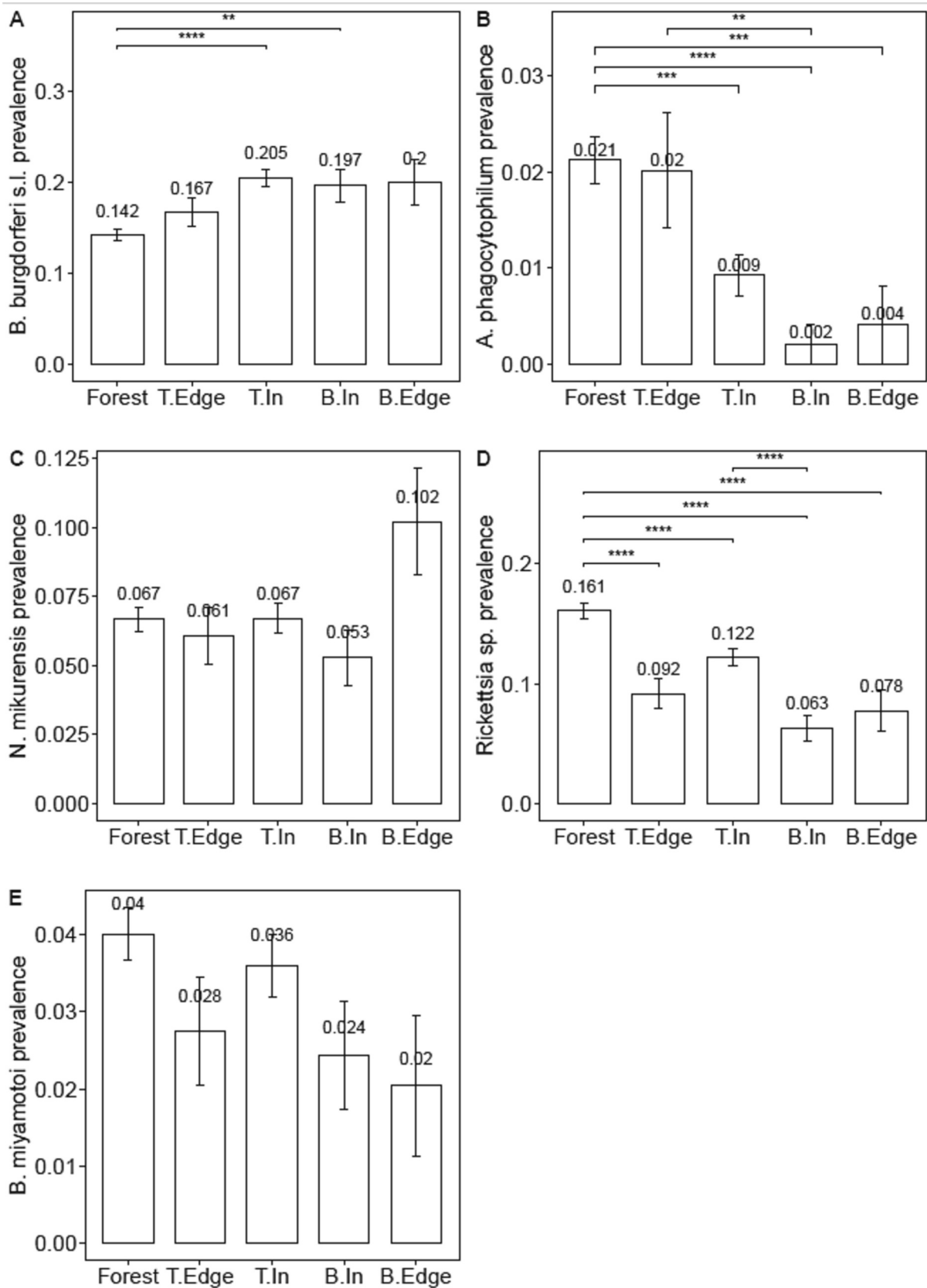


Fig. 6. Mean \pm se infection prevalence for each screened pathogen per location type (Forest = Forest stand interior, T.In = interior trail, T.Edge = edge trail, B-In = interior bench and B.Edge = edge bench). Pathogens include (A) *Borrelia burgdorferi* s.l.; (B) *Anaplasma phagocytophilum*; (C) *Neorhlichia mikurensis*; (D) *Rickettsia helvetica* and (E) *Borrelia miyamotoi* (Please note the differences in scale of the Y-axes). The significance of the differences in NIP between location types are quantified as p-values of associated pairwise t-tests (ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

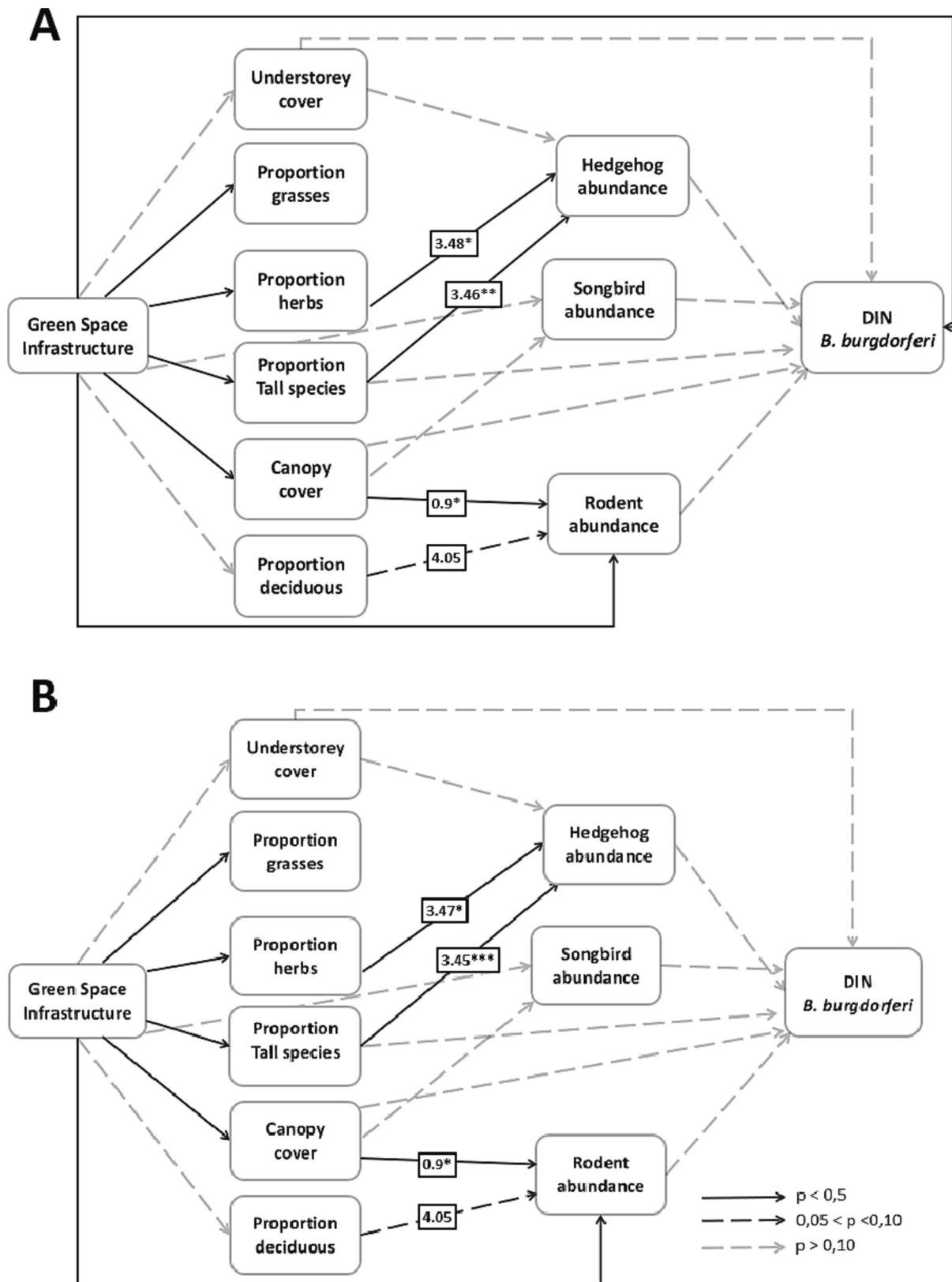


Fig. 7. Fitted structural equation models for the density of questing infected nymphs (DIN) visualizing the pathways that were expected to be significant based on GLMM results (“Significance models”). The boxes on all significant paths display estimate coefficients and significance levels. *, ** and *** denote significance levels with p -values lower than 0.05, 0.01 and 0.001, respectively. A) significance model including a direct effect of green space infrastructure on DIN and B) significance model excluding a direct effect of green space infrastructure on DIN.

trails demonstrates that the higher NIP at trails compensates for the comparatively lower DON, establishing a measurable effect on the relative distribution of DIN within forest stands. The trend towards positive correlation of local songbird abundance with both DON and NIP (specifically for *B. garinii*) can be assumed to be a result of the songbirds' role as reservoir hosts. This near-correlation is further reflected in the trend towards a positive correlation with DIN. Similarly, the significant correlation of canopy cover with DON and NIP (*B. afzelii*) persists in a significantly positive effect on DIN.

4.2. Density of questing nymphs

The observed variation in DON between location types, with the highest density being measured in the forest interior, is likely associated with the observed differences in vegetation characteristics and local host abundance. Higher values for cover in both understorey and canopy, rodent and deer density were observed in the forest interior, all of which have been associated with higher DON in previous research (Hofmeester et al., 2017b; Hofmeester et al., 2016; Krawczyk et al., 2020; Schulze and Jordan, 2005; Tack et al., 2013). Additionally, DON was observed to be on average twice as high at trails in the forest interior, compared to trails at the forest edge (0.61 ± 0.20 and 0.28 ± 0.11). This observation is in line with higher canopy cover and local abundance of potential larval hosts in interior locations compared to forest edge (specifically songbirds, hedgehogs and squirrels, Fig. S.5). Although no significant difference in local rodent density was observed between trail and bench types, the significantly higher density in forest interior implies either a micro-scale edge effect, possibly based on avoidance behaviour towards humans (Ecke et al., 2002; Miklós and ĩiak, 2002). The edge effect with regards to rodents and its effect on DON has been implied in previous work also, where the density of the wood mouse *Apodemus sylvaticus*, a prominent larval host, was observed to correlate positively with woodland cover (Perez et al., 2016).

The higher DON in forest interior is likely driven in large parts by the local abundance of propagation hosts (hereafter "roe deer", the most abundant propagation hosts in the study region). Although our camera trapping setup is not optimal for the detection of large mammals, the observation that roe deer spend most of their time in the forest interior was also made in the recent literature (Myserud, 1996; Tufto et al., 2020). The resulting spatial variation in DON has been described recently by Mols et al. (2022). These observations on a within-forest stand scale are in contrast to the landscape level observation that the amount of edge habitat positively affects DON by the provision of suitable foraging habitat for large ungulates (Tack et al., 2012b). This underlines the different mechanisms at play between total host population density and their habitat use (here: "local abundance"). We therefore stress that applying insights in tick and pathogen ecology, gathered from landscape scale research, to micro-geographic green space management may not always be straightforward.

We also observed an effect of local host abundance and vegetation on the variation in tick density between plots. For example, locations where rodents and songbirds spend more time were associated with higher DON. This again highlights that the role of TBD hazard drivers should be considered on different spatial scales: we demonstrate the relevance of their habitat use within forest stands as well as differences in abundance between forest stands. In this case, these observations are in line with other studies describing similar effects on between-forest stand scales or even landscape scales (Hillyard, 1996; Hofmeester et al., 2016; Krawczyk et al., 2020; Sonenshine, 1991). The observed positive association of DON with understorey cover and the proportion of tall species within this understorey is also in line with observation on larger spatial scales, as it is well-documented that more structure rich vegetation is associated with higher tick density (Ehrmann et al., 2017; Tack et al., 2013; Tack et al., 2012a; Verheyen et al., 2021). This vegetation cover effect may be due to local host abundance being affected positively, as canopy cover has been shown to correlate with the density of rodents, both in this

study and in previous research (Ecke et al., 2002; Krawczyk et al., 2020). In addition, higher understorey cover and more tall understorey species, such as tree saplings and shrubs, create both favourable browse and covert resting sites for roe deer (Myserud, 1996; Myserud et al., 1999; Tixier and Duncan, 1996). These vegetation characteristics and the associated microclimate variation, both temporal and spatial, may also have an effect on the questing behaviour of *I. ricinus* (Mejlon and Jaenson, 1997; Perret et al., 2000) and its long-term survival (Dautel et al., 2016; Elias et al., 2006; Van Gestel et al., 2022). The former results in drag sample measurements not being a representation of the total population density, but a means to compare locations subjected to similar sampling effort with respect to tick bite risk both for humans and wildlife (Nyrh   et al., 2020; Tack et al., 2011), as ticks that do not quest are ecologically irrelevant.

4.3. Pathogen prevalence

The nymphal infection prevalence (NIP) of *Borrelia burgdorferi* s.l. was observed to vary significantly between months and years, with overall higher values from July onwards. The higher NIP in summer is in accordance with recent observations in the Netherlands, although we did not observe a similar decrease in NIP in autumn (Hartemink et al., 2021). NIP was also significantly higher at infrastructure, compared to the forest interior. This implies that a local dilution step is established, as a smaller proportion of larvae feeds on infectious hosts: either by feeding on an uninfected reservoir host or feeding on an incompetent host. The former could be the case since the local abundance of mice was observed to be considerably higher in the forest interior (about 5 times), potentially increasing the chances of a larva feeding on an uninfected mouse. An additional dilution step may be facilitated by the habitat use of roe deer, which are incompetent hosts for *B. burgdorferi* s.l. (Jaenson and T  lleklint, 1992; Telford 3rd et al., 1988), and spend most of their time in the forest interior. Consequently, a proportion of larvae will feed on uninfected hosts (rather than on *B. burgdorferi* infected rodents, hedgehogs or songbirds). This hypothesis is supported by the observation that the second lowest NIP was recorded at edge trails, the only other location type, besides forest interior, where roe deer were sighted. It is to be expected they pass by regularly to browse in adjacent ecotones and meadows, whilst avoiding benches (Borowski et al., 2021). These associations of NIP with roe deer habitat use are in line with the observed, inverted patterns in the NIP of *Anaplasma phagocytophilum*, which is highest in forest interior and at edge trails. Roe deer are the main reservoir host for ecotype 2 of this bacterium, which explains how more larvae in the interior and edge are infected, as the chances of feeding on an infected host are higher (Fabri et al., 2022; Jahfari et al., 2016; Stuen et al., 2013). The slightly higher NIP at interior trails compared to benches may be due to a higher local abundance of hedgehogs, which are reservoir hosts for ecotype 1 of *A. phagocytophilum* (Jahfari et al., 2014). The positive association with canopy cover is likely due to more closed canopy providing more suitable habitat for roe deer, whereas the negative association with the proportion of deciduous vegetation in the canopy may be due to its positive association with rodent abundance: as rodent density increases, a lower proportion of larvae will feed on roe deer. Our results represent the first detection of ecotype 1 and 2 of *A. phagocytophilum* in questing nymphs from Belgium, to our knowledge.

The prevalence of *Borrelia afzelii*, the most prevalent genospecies of *B. burgdorferi*, exhibits a similar pattern to that of *B. burgdorferi* s.l., suggesting that this genospecies is the main determinant in the dilution steps described above. In addition, local vegetation characteristics seem to be determining factors of its NIP, whereas no effects of larval host abundance, additional to the infrastructure effect, were observed. The positive effect of the proportion of tall understorey species and canopy cover may be due to differences in survival based on infection with *B. afzelii*: a positive infection status has been shown to enhance tick survival in challenging conditions (Herrmann and Gern, 2010). This implies that ticks infected with this genospecies might optimally profit

from the enhanced microclimate buffering provided by vegetation, increasing their chances of surviving until attachment to a host and thereby furthering the *B. afzelii* life cycle, increasing overall prevalence. Additionally, the positive correlation of the local abundance of two groups of reservoir species (rodents and hedgehogs) with the vegetation characteristics in question (canopy cover and the proportion of tall understorey species, respectively) may lead to an indirect spatial sorting through host habitat use, although no correlations between NIP and local host abundance were found to be significant. The negative correlation with deciduous vegetation, or rather the positive one with coniferous vegetation, is in line with observations made by [Ruyts et al. \(2016\)](#) on a landscape scale.

For the NIP of *B. garinii*, no significant infrastructure or vegetation effects were observed. This is explained by the observation that the local abundance of songbirds, the main reservoir species group ([Heylen et al., 2014](#)), did not correlate with any explanatory variables (the slightly higher abundance of songbirds and NIP of *B. garinii* at interior trails was not significant). Nonetheless, the expected positive correlation with local songbird abundance ([Hubalek et al., 2006](#)) was confirmed. In addition, negative estimates for the local abundance of pigeons were found. This is in line with the statement that pigeons are undesirable hosts for both *I. ricinus* and *B. garinii* ([Fabbi et al., 1995](#)). Therefore, we postulate that the composition of the local bird community that frequents a certain location drives the nymphal infection prevalence of *B. garinii*.

The lack of significant differences in the NIP of *Borrelia miyamotoi* and *Rickettsia* sp. may be due to their vertical transmissibility. For pathogens that are vertically transmissible survival of its vectors, that also act as reservoir hosts ([Sprong et al., 2009](#)), will affect the infection prevalence ([Fine, 1975](#); [Lipsitch et al., 1996](#)). Differences in survival within and between comparable forest stands are likely too small to create significant differences in the NIP of these pathogens ([Van Gestel et al., 2022](#)).

4.4. SEMs and implications

We attempted to draft the correlations described above in a causative network through the use of structural equation models (“SEMs”). The considerable decrease in AICc between the full conceptual models (for DIN, DON and NIP of *B. burgdorferi* s.l.) and the ones built based on significant correlations (“significance models”) indicates that the latter are a better fit to the data, as they omit insignificant pathways. The significance of effects on DON is dependent on the inclusion of the location type factor, which indicates that part of the variation in DON is still explained by factors correlated to green space infrastructure that are not included in the model, such as the local abundance of roe deer. The latter could be quantified in future research by supplementing the current camera trapping approach with conventional camera trapping of large hosts ([Hofmeester et al., 2017a](#)), mapping ungulate bed-sites ([Myrsterud, 1996](#)) or the relative density of deer droppings ([Mols et al., 2022](#)). The performance of the models, and therefore the estimation of indirect effects of infrastructure and vegetation through the alteration of host habitat use, could be increased by an improved sample size for local host abundance (i.e. more trapping sessions).

5. Conclusions and application

This study is, to our knowledge, the first to try and analyse vegetation and host effects on the distribution of ticks and tick borne pathogens both within and between green spaces and forest stands. Our results are in support of dilution/inhibition steps to be affecting the infection prevalence of *Borrelia burgdorferi* s.l. and its genospecies on a within-forest stand scale (40 m). Herein, dilution steps for *B. burgdorferi* s.l. and *B. afzelii* are associated with the expected abundance of potential dilution hosts (roe deer). On a slightly larger scale differences in NIP of *B. garinii* between forest stands correlate positively with the abundance

of reservoir hosts (songbirds) and negatively with the abundance of expected dilution hosts (pigeons). On both these scales, variation in disease risk is mainly a function of tick density, although the significant variation in NIP can affect the relative hazard between nearby locations. The vegetation characteristics correlating with both the NIP of *B. afzelii* and rodents, its reservoir hosts, provide an additional, indirect argument in favour of the amplification/inhibition steps at this scale. These observations bring additional nuance to the observations made in past work at different spatial scales, where no support for a dilution effect with regards to *B. burgdorferi* s.l. was found in studies comparing green spaces ([Ruyts et al., 2016, 2018](#)). We stress the need to not only study the effects of the drivers of DIN/DON/NIP on landscape scales, but consider the impact of micro-geographic variation on disease hazard.

With respect to green space management, our results stress the relevance of zoning (i.e. dividing the total area in smaller zones and assigning priority functions/ecosystem services). When deciding the focus of a zone, our results allow to estimate and take into account the expected relative tick bite/disease hazard. It may, for example, be more desirable to assign a recreational purpose to forest stands with less developed shrub layers or more open canopies. They are expected to harbour less (propagation and larval) hosts and therefore ticks. From a similar point of view, focussing visitor flows at edge trails rather than trails in the forest interior will considerably lower TBD hazard. For green spaces where such planning is not possible or desirable, our results allow to better estimate TBD risk and plan small-scale management efforts accordingly. Depending on the ecosystem service that is prioritized within a zone, the local management strategy may be attuned to reduce TBD risk: areas where high tick densities and recreational pressure are expected may warrant repeated management that focuses on contact rate reduction or visitor sensibilisation. Given the small scale nature of the observed effects, we consider it relevant to apply management efforts to green space infrastructure where visitors are exposed, even if it is not desirable to apply them to the entirety of the surrounding forest stand. In this respect, mowing or sheep mopping may be performed at high-risk infrastructure when high tick activity is expected (predominantly spring), without jeopardizing management goals such as biodiversity and nature experience on the forest stand level.

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CRediT authorship contribution statement

Mats Van Gestel: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dieter Heylen:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Kris Verheyen:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Manoj Fonville:** Methodology, Investigation, Data curation, Conceptualization. **Hein Sprong:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Erik Matthysen:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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