

RESEARCH ARTICLE

Sand lizards (*Lacerta agilis*) decrease nymphal infection prevalence for tick-borne pathogens *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in a coastal dune ecosystem

Clara Florentine Köhler¹  | Hein Sprong¹ | Manoj Fonville¹ | Helen Esser² | Willem Frederik de Boer² | Vincent van der Spek³ | Annemarieke Spitzen-van der Sluijs^{4,5}

¹Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

²Wildlife Ecology and Conservation Group, Wageningen University, Wageningen, The Netherlands

³Department Bron- en Natuurbeheer, Waternet, Amsterdam, The Netherlands

⁴Reptile, Amphibian and Fish Conservation the Netherlands, Nijmegen, The Netherlands

⁵Institute for Water and Wetland Research, Animal Ecology and Physiology, Radboud University, Nijmegen, The Netherlands

Correspondence

Clara Florentine Köhler
Email: clara.kohler@rivm.nl

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Abstract

1. Understanding which factors determine tick-borne disease hazard can contribute to effective disease control. In Europe, the hazard of the pathogens *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum* is determined by local tick densities (mainly *Ixodes ricinus*) and the reservoir competence of the host species community. Sand lizards (*Lacerta agilis*) are common hosts for larvae and nymphs of *I. ricinus* and non-competent reservoirs for both pathogens. Consequently, high relative abundance of *L. agilis* is hypothesized to be associated with lower infection prevalence in nymphs. Here, we aimed to test whether this effectively occurs in natural settings.
2. We sampled different habitat types within a heterogeneous dune landscape at the Dutch coast and estimated (1) *L. agilis* densities, (2) host community competence, (3) the density and infection prevalence of questing *I. ricinus* ticks and (4) the number and infection prevalence of ticks feeding on *L. agilis*.
3. Captured *L. agilis* had high tick burdens and contributed substantially to feeding *I. ricinus* larvae in their natural habitat. *B. burgdorferi* s.l. and *A. phagocytophilum* were virtually absent from feeding larvae and nymphs.
4. The nymphal infection prevalence of both pathogens in questing ticks was lower in habitat types where *L. agilis* was more abundant. Hence, *L. agilis* strongly reduced community competence.
5. The density of questing nymphs was higher in habitat types with denser vegetation and also varied more between habitat types than infection prevalence. As a result, nymphal density had a stronger effect on the density of infected ticks than did nymphal infection prevalence.
6. *Synthesis and applications.* Coastal dune habitats favourable for *L. agilis* have lower densities of questing nymphs and a lower human infection hazard. These results might be applicable to similar ecosystems where *L. agilis* is present. From

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a public health perspective, this underlines the importance of preserving early successional habitat, as encroaching shrubs are associated with higher tick-borne disease hazard, and vegetation removal might be a solution to reduce hazard in coastal dunes. The high degree of spatial heterogeneity in the abundance of tick-borne pathogens also poses opportunities to manage recreational activities to limit human exposure to tick-borne diseases.

KEYWORDS

community competence, dilution host, Europe, habitat suitability, *Ixodes ricinus*, Lyme borreliosis, reservoir, vector-borne diseases

1 | INTRODUCTION

A key element in preventing tick-borne diseases is understanding which factors determine hazard to humans (Swei et al., 2020). In Europe, *Ixodes ricinus* ticks are important vectors of various pathogens of veterinary and public health concern (Heyman et al., 2010). Two pathogens that are frequently found in *I. ricinus* are bacteria of the *Borrelia burgdorferi* species complex and *Anaplasma phagocytophilum*. An infection with *B. burgdorferi* sensu lato causes Lyme borreliosis in humans, the most frequently transmitted tick-borne disease in both Europe and North America. Infection with *A. phagocytophilum* may cause human granulocytic anaplasmosis but is rarely diagnosed or reported for various reasons, among others the presence of non-characteristic “flu-like” symptoms and a lack of effective laboratory diagnostic tools (Matei et al., 2019). *A. phagocytophilum* is also of veterinary importance, causing tick-borne fever in both wild and domestic ruminants and in dogs. In Europe, the transmission dynamics of both pathogens relies on horizontal transmission between *I. ricinus* ticks and wildlife reservoir species (Hauck et al., 2020). An important eco-epidemiological question is how changes in wildlife community composition affect the densities of infected ticks, and hence tick-borne disease hazard.

As humans are most frequently infected by the nymphal stage of *I. ricinus*, the local infection hazard is quantified as the density of infected nymphs (DIN). DIN is the product of two factors: the density of questing nymphs (DON) and the nymphal infection prevalence (NIP). As *I. ricinus* ticks need vertebrate blood in all three life stages (Földvári, 2016), DON depends on the densities of suitable host species. The immature stages of *I. ricinus* feed on a wide variety of species, including small to medium-sized mammals, reptiles and ground-foraging birds. Adult stages almost exclusively feed and propagate on larger mammals, such as roe deer. The presence and density of these species affects DON (Hofmeester et al., 2017). *I. ricinus* spends more than 90% of its life cycle in the vegetation. To maintain homeostasis and prevent dehydration during the long off-host periods, favourable microclimatic conditions are crucial for tick survival and activity. As a consequence, *I. ricinus* is restricted to forests, shrub and grassland with dense vegetation, where humidity at ground level is >80%. (Estrada-Peña et al., 2013).

While the availability of host species is essential for tick survival and, thus, affects DON, the relative densities of these species within

the host community play a key role in pathogen transmission dynamics, and hence NIP. Host species differ considerably in their reservoir competence, that is, the ability of a species to effectively maintain and transmit a pathogen to uninfected ticks. Realized reservoir competence also considers the species-specific average individual tick burden, an indication of the number of ticks successfully feeding on the host (Brunner & Ostfeld, 2008). Competent reservoir species for *B. burgdorferi* s.l. are rodents and ground-foraging birds (Hofmeester et al., 2016). In contrast, the main reservoir hosts for *A. phagocytophilum* in Europe are ungulates and medium-sized mammals like the European hedgehog *Erinaceus europaeus* (Fabri et al., 2021; Silaghi et al., 2012). The relative contribution of these host species to feeding ticks determines the infection prevalence. Local variation in host species assemblage composition is, therefore, likely to influence tick-borne disease hazard (Krawczyk et al., 2020).

While high relative densities of competent reservoir hosts increase NIP in questing *I. ricinus*, incompetent species can have the opposite effect. Here, special attention should be directed at the role of lizards, as the majority of lizard species appear to be refractory to infection with most *B. burgdorferi* genospecies, the Mediterranean variant *B. lusitaniae* being the exception (Lane, 1990; Majláthová et al., 2006; Tjisse-Klasen et al., 2010). Where *B. lusitaniae* is absent, such as Central and Western Europe, lizards may function as dilution hosts, so that NIP should be lower in species assemblages where lizards feed a large proportion of larvae. Several studies have been conducted to investigate this effect, focusing on different geographical scales and lizard species. Some studies have found a negative correlation between lizard density and NIP, whereas others have found no correlation (Mannelli et al., 2012; Ostfeld & Keesing, 2000; Tjisse-Klasen et al., 2010; Wright et al., 1998). It remains unclear what the eco-epidemiological role of lizards is in the transmission of tick-borne pathogens. The contradictory nature of previous findings may be due to differences in host species community that were not accounted for. Because the role of lizards as hosts for *I. ricinus* larvae is always relative to the role of other host species in the same area, it is essential to consider the composition of the local host species assemblage as a factor in the analysis.

Here, we quantify the role of sand lizards (*Lacerta agilis*) as a potential dilution host for *B. burgdorferi* s.l. and *A. phagocytophilum* in a heterogeneous landscape, while taking into account local community competence (CC). *L. agilis* is an important host for larvae and

nymphs of *I. ricinus* in central and Western Europe, while being an incompetent host for both *B. burgdorferi* s.l. and *A. phagocytophilum* (Tijssen-Klasen et al., 2010). We sampled questing *I. ricinus* from the vegetation across six different habitats along a successional gradient in a coastal dune ecosystem associated with varying densities of *L. agilis*. Per habitat type, we calculated CC based on the estimated density and tick burden of *L. agilis* and other relevant host species. Furthermore, we estimated DIN for each habitat type based on tick density and infection prevalence to show the spatial variation in tick-borne disease hazard in a mosaic landscape. To accurately determine local tick burden of *L. agilis* and confirm its reservoir incompetence, we tested feeding ticks collected from local *L. agilis* for our focal pathogens. To our knowledge, this study is the first to assess the role of *L. agilis* as a dilution host in the context of local CC for *B. burgdorferi* s.l. in the field and the first to link local prevalence of *A. phagocytophilum* to the abundance of lizards. This is a key step to further understand how these pathogens persist in local wildlife assemblages and which biotic factors determine tick-borne disease hazard.

2 | MATERIALS AND METHODS

2.1 | Field data collection

2.1.1 | Study area

Fieldwork was conducted between May and September 2021 in the Amsterdam Water Supply Dunes, located at the Dutch North Sea coast (Figure 1). Permission to enter and collect samples was

granted by Waternet, on behalf of the municipality of Amsterdam. Most of the area consists of fixed grey dunes, partly covered with *Hippophae rhamnoides* shrubland and dune forests. The host species community for *I. ricinus* is relatively limited. *Dama dama* are the only ungulate species in the area, as roe deer (*Capreolus capreolus*) have disappeared almost entirely. The only rodent species found in significant densities are wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*). *Lacerta agilis* is the only reptile species present. Six main habitat types can be distinguished: forest (closed canopy), dense shrub (with high amount of undergrowth), bracken (*Pteridium aquilinum* vegetation outside of forests), open forest (sparse canopy), grassland (herbaceous vegetation with occasional trees and shrubs) and open shrub (sparse shrub and herbs). See Appendix S1 for a detailed description of the habitat types.

2.1.2 | Collection of questing *Ixodes ricinus* from vegetation

In total, we collected questing nymphs from 83 sites (forest: $n = 23$, dense shrub: $n = 15$, bracken: $n = 3$, open forest: $n = 14$, open shrub: $n = 18$, grassland: $n = 10$). Sampling points were selected using a stratified random sampling approach. Location and sequence of sampling points were assigned using a GIS random sampling tool (QGIS.org, 2021). We maintained a minimum distance of 100m between sampling points and 20m to the nearest edge of the habitat type to account for possible edge effects. Tick collection only took place on days without rain and air temperatures above 10°C (Perret et al., 2000). At each site, DON was determined by dragging a 1 m² white cloth across a transect of

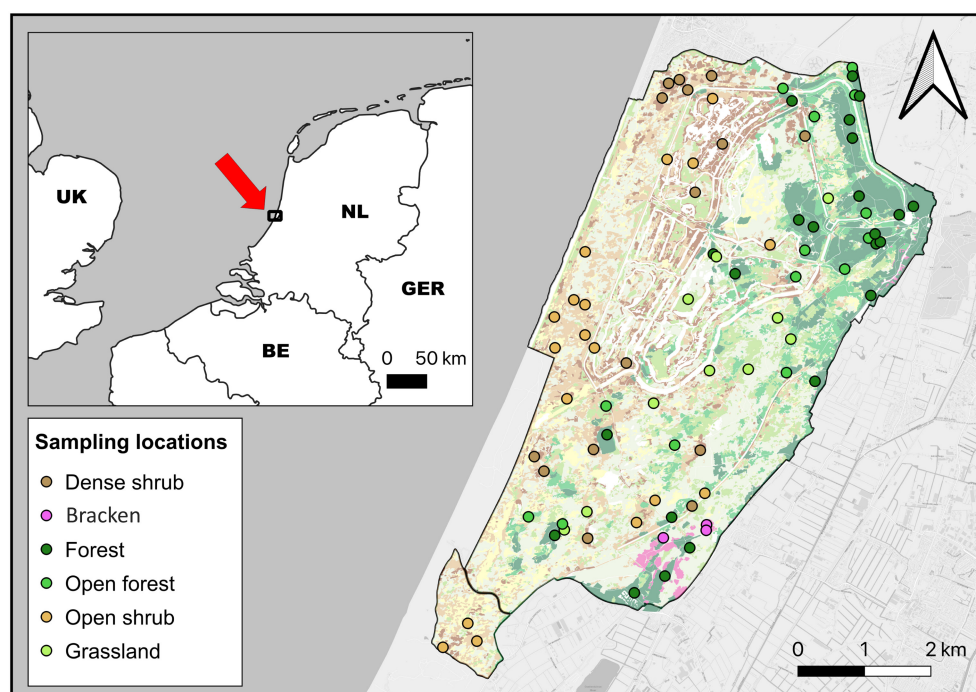


FIGURE 1 Reference map of the study area. Points indicate the placement of sampling points within the area.

100 m (Gray & Lohan, 1982). At 5 m intervals, nymphs and adults attached to the fabric were collected. Larvae were counted but not collected. Because the transects did not always generate sufficient ticks to estimate NIP, we continued sampling around the transects until at least 100 individual nymphs were collected or until 2 h of blanket-dragging had passed. This additional sampling did not count towards our estimates for DON.

2.1.3 | Collection of ticks from *Lacerta agilis*

We regularly searched for *L. agilis* in suitable habitat throughout the entire study area at and around all transects. Individual lizards were captured either by hand or with a noose, taking care to handle the animal cautiously to prevent autotomy of the tail. Adults were sexed by visual inspection, for subadults we followed the method of Eplanova & Roitberg, 2015. Lizards were weighed after tick removal, using a precision scale (On Balance Tuff-Weigh 100×0.01 g). Larvae and nymphs were removed using forceps and stored individually in 0.2 mL Eppendorf PCR tubes. All lizards were released immediately after the procedure. All methods involving animals were approved by and carried out in accordance with the guidelines and regulations of permit AVD261002016604 issued by the ethical committee of RAVON and permit FF/75A/2016/015, issued by the Netherlands Enterprise Agency.

2.2 | Estimating community competence

2.2.1 | Determining species assemblage

In addition to *L. agilis*, 10 other species (five mammals and five birds) were included in the analysis as relevant hosts for *I. ricinus* larvae (Table 1). We combined information from literature and local species monitoring datasets to estimate the relative densities of host species per habitat type (Table S2.1). For all species except *L. agilis*, we retrieved values for larval burden and pathogen prevalence in feeding larvae from a recent metanalysis of the literature by Fabri et al. (2022) and Hofmeester et al. (2016). See Appendix S2 for more details.

2.2.2 | Calculation of community competence and realized community competence

To estimate CC, we followed the framework of Hofmeester et al. (2016), which is an adaptation of the equations in Mather et al. (1989). Relative importance (RI_i) of each host species in feeding *I. ricinus* larvae was calculated using Equation (1); where D_i is the population density, LB_i the mean individual larval burden and $\sum_j^n D_j LB_j$ the total amount of larvae fed by the host community. CC is calculated using Equation (2). Multiplying the reservoir competence of each species (RC_i) with LB_i and D_i yields the total number of larvae

infected by each species. CC is the sum of all infected larvae divided by the total number of fed larvae.

$$RI_i = \frac{D_i \times LB_i}{\sum_j^n D_j \times LB_j}, \quad (1)$$

$$CC = \frac{\sum_i^n D_i \times LB_i \times RC_i}{\sum_j^n D_j \times LB_j}. \quad (2)$$

Local CC was calculated for each of the six habitat types, representing the proportion of fed larvae that become infected given the local species assemblage. We then calculated realised community competence (RCC; Equation 3), which reflects the absolute number of fed larvae that become infected and is theoretically comparable with DIN. Division by 10,000 is necessary to scale RCC from ind./km² to ind./100m² to be able to compare it with measured DIN.

$$RCC = \frac{\sum_i^n D_i \times LB_i \times RC_i}{10,000}. \quad (3)$$

2.3 | Data analysis

We screened individual ticks for *B. burgdorferi* s.l. and *A. phagocytophilum*. Positive samples for *B. burgdorferi* s.l. were subsequently analysed to genotype, samples positive for *A. phagocytophilum* were analysed to ecotype (Jahfari et al., 2014). Ticks collected from *L. agilis* were also tested for *Borrelia miyamotoi*, *Neorhlichia mikuriensis*, *Babesia microti* and *Spiroplasma ixodetis*. See Appendix S2 for a description of the molecular protocols used. All statistical analyses were performed using R version 4.1.2 (R Core Team, 2021). We used QGIS for spatial data analysis (QGIS.org, 2021). Hourly measurements of saturation deficit (SD) were obtained from the Royal Netherlands Meteorological Institute (KNMI, 2022a). We averaged data of the two weather stations closest to the study area: Wijk aan Zee (~18 km) and Voorschoten (~24 km).

We used a negative-binomial generalized linear model (GLM) with a log-link to test the effects of habitat type, day of year (to account for seasonality), weight and sex on individual tick burden of *L. agilis* (larvae and nymphs combined). Similarly, a negative-binomial GLM with a log-link was used to test for an effect of habitat type and possible confounding effects of SD and day of year on DON (n/100m²) at each sampling site. To test whether NIP in questing ticks was associated with CC, we used binomial generalized linear mixed modelling (GLMM) to analyse the log-likelihood of nymphs testing positive for either pathogen. Transect was included as a random effect. Models including possible confounding effects of day of year and the CC of surrounding habitat (100-m radius) on NIP are included in Appendix S4. We used packages MASS for GLMs (Venables & Ripley, 2002) and lme4 for GLMMs (Bates et al., 2015). Estimated marginal means (EMMs) and confidence intervals (CIs)

TABLE 1 Mean larval burden and reservoir competence of host species included in the analysis.

	Mean larval burden (n) ^a	Reservoir competence (n) ^b	
		<i>Borrelia burgdorferi</i> s.l.	<i>Anaplasma phagocytophilum</i>
Reptiles			
<i>Lacerta agilis</i>	10.24 (100)	0.002 (1031)	0.005 (1031)
Mammals			
<i>Apodemus sylvaticus</i>	6.67 (3448)	0.295 (2738)	0.009 (109)
<i>Dama dama</i>	0.41 (102)	0.042 (48)	0.79 (42) ^c
<i>Erinaceus europaeus</i>	108.52 (625)	0.276 (431)	0.258 (31) ^d
<i>Myodes glareolus</i>	3.82 (9700)	0.264 (3954)	0 (158)
<i>Sciurus vulgaris</i>	24.81 (26)	0.284 (366) ^d	N/A
Birds			
<i>Fringilla coelebs</i>	1.13 (646)	0.016 (123)	N/A
<i>Parus major</i>	0.55 (591)	0.093 (613)	0 (243)
<i>Troglodytes troglodytes</i>	0.36 (111)	0.006 (158)	N/A
<i>Turdus merula</i>	3.1 (467)	0.114 (821)	0.029 (35) ^d
<i>Turdus philomelos</i>	2.44 (305)	0.216 (366)	0 (30) ^d

^aNumber of individual hosts analysed.

^bNumber of larvae screened for pathogens.

^cBased on Fabri et al. (2021).

^dReservoir competence based on infection prevalence in hosts rather than feeding larvae (n represents the number of hosts analysed).

were retrieved using the *emmeans* package (Lenth et al., 2022). We used package *DHARMa* to test model assumptions for all GLMs and GLMMs (Hartig & Lohse, 2022). All graphs were made using *ggplot2* (Wickham, 2016).

3 | RESULTS

3.1 | Feeding ticks

3.1.1 | Tick burden of *Lacerta agilis*

We captured a total of 100 individual *L. agilis* (May: $n = 17$, June: $n = 30$, July: $n = 31$, August: $n = 20$, September: $n = 2$). Of the captured individuals, 49 were female, and 51 were male. Tick infestation occurred on 93 out of 100 lizards, of which 24 individuals were infested with larvae, but not with nymphs. None of the lizards were infested with adult ticks. All feeding ticks were identified as *I. ricinus*. The highest observed tick burden was 51 ticks on an adult female. Mean larval burden was 10.4, while mean nymphal burden was 3.2. Likelihood-ratio tests showed that individual tick burden was dependent on habitat type ($\chi^2 = 17.28$ (3), $p < 0.001$) and sex ($\chi^2 = 12.76$ (1), $p < 0.001$). Body weight ($\chi^2 = 3.53$ (1), $p = 0.06$) and day of year ($\chi^2 = 0.37$ (1), $p = 0.54$) were nonsignificant. Tick burden was highest in individuals captured near bracken (EMM = 20.62 ± 4.10) and lowest in individuals captured in grassland (EMM = 5.22 ± 1.39). Tick burden was more

than twice as high in males (EMM = 16.58 ± 2.41) compared with females (EMM = 7.79 ± 1.23 , Table S3.1; Figure S3.1).

3.1.2 | Pathogens detected in ticks feeding on *Lacerta agilis*

We collected 1038 larvae and 324 nymphs feeding on *L. agilis*, of which 1031 larvae and all nymphs were successfully analysed. Only two larvae and one nymph were infected with *B. burgdorferi* s.l., each collected from a different individual. *A. phagocytophilum* was detected in five larvae and two nymphs, collected from four different lizards. Results for other pathogens are included in Table S3.2.

3.2 | Community competence per habitat type

3.2.1 | Relative importance of host species

The relative importance of *L. agilis* in feeding larvae was largest in grassland and open shrub (Figure 2a). In both habitat types, they accounted for more than 80% of all fed larvae. In less suitable habitat types for *L. agilis*, such as dense shrub and forest, rodents accounted for the highest proportion of fed larvae. Other vertebrates were of minor importance in comparison with *L. agilis*, *A. sylvaticus*, *M. glareolus* and *E. erinaceus*.

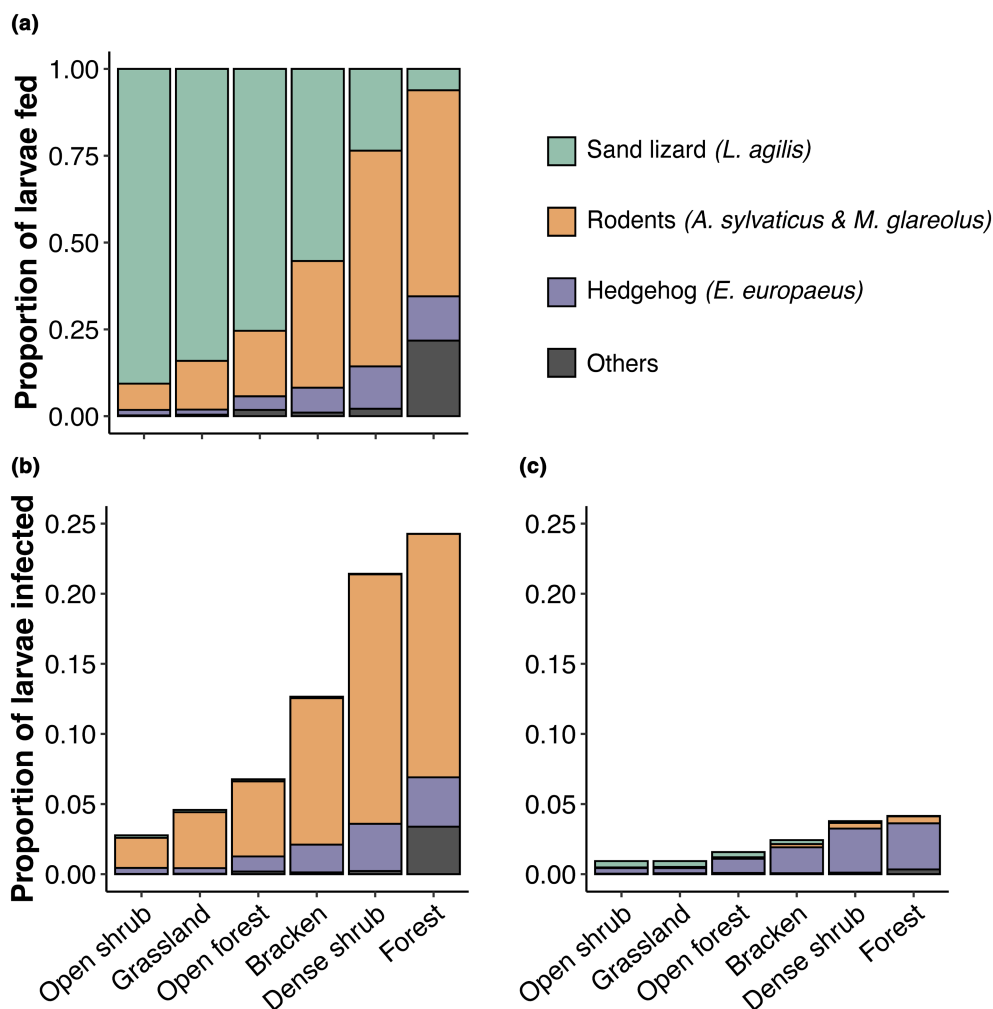


FIGURE 2 Upper bar plot (a) shows the contribution of each host species to feeding *Ixodes ricinus* larvae in the different habitat types. Lower plots show the proportion of larvae infected with (b) *Borrelia burgdorferi* s.l. or (c) *Anaplasma phagocytophilum* and the contribution of different host species to infecting larvae. 'Others' represents all other host species included in the analysis (Table 1).

3.2.2 | Community competence

The estimated CC (i.e. the proportion of larvae infected by the community at any given time) for both pathogens was highest in forest and dense shrub, and lowest in grassland and open shrub (Figure 2b,c; Table S4.1). Estimated CC for *A. phagocytophilum* was overall lower than for *B. burgdorferi* s.l. While rodents were the most important reservoir for *B. burgdorferi* s.l., *A. phagocytophilum* in nymphs originated primarily from *E. erinaceus* (Figure 2b,c).

3.2.3 | Realized community competence

Besides CC, we also quantified RCC, which is the predicted number of infected larvae per 100m², theoretically comparable with DIN (Table S4.1). Bracken and dense shrub had the highest estimated RCC for *B. burgdorferi* s.l., whereas all other habitat types were expected to produce less infected larvae (Figure 4c). Estimates for RCC of *A. phagocytophilum* were overall lower but also highest in bracken and dense shrub (Figure 4d).

3.3 | Questing ticks

3.3.1 | Density of questing nymphs

DON, as determined by blanket-dragging, ranged from 0 to 103 individuals per 100m² (Figure 3). DON was dependent on habitat type ($\chi^2 = 60.67$ (5), $p < 0.001$) and decreased with day of year ($\chi^2 = 22.01$ (1), $p < 0.001$; $\beta = -0.02 \pm 0.004$, $p < 0.001$), but not on SD ($\chi^2 = 0.04$ (1), $p = 0.84$). Nymphs were most abundant in bracken (EMM = 34.65 ± 21.27) and least abundant in open shrub (EMM = 1.11 ± 0.40 ; Tables S4.2 and S4.3).

3.3.2 | Nymphal infection prevalence

In total, 4336 questing nymphs were successfully tested for the presence of both tick-borne pathogens. Of those, 320 samples were positive for *B. burgdorferi* s.l. (7.38%), and 162 samples tested positive for *A. phagocytophilum* (3.76%). We calculated NIP as the arithmetic mean infection prevalence per habitat type (Table S4.1).

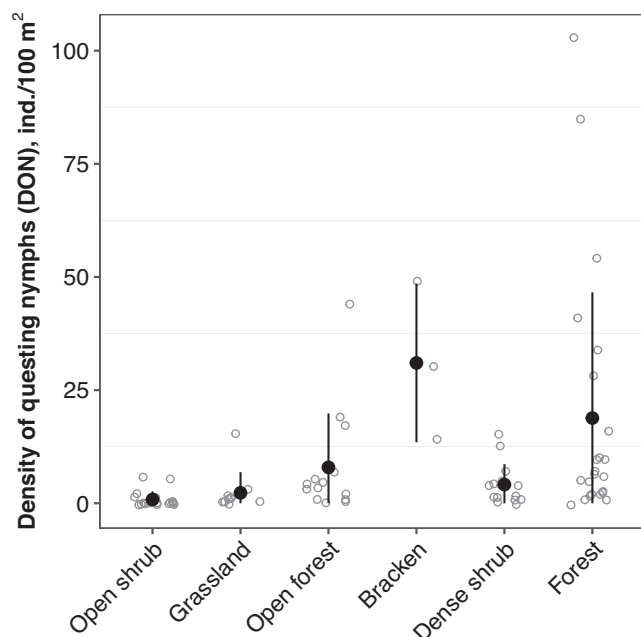


FIGURE 3 Mean and standard deviation of the density of *Ixodes ricinus* nymphs ($n/100\text{ m}^2$) per habitat type. Original measurements are added as grey dots for reference.

Neither day of year nor CC of surrounding habitat had any effect on measured NIP and were, therefore, not included in the model (Tables S4.7–S4.8). NIP for *B. burgdorferi* s.l. was highest in dense shrub, about twice as high as in forest. Open forest and grassland had the lowest measured NIP (Figure 4a). The log-odds for a sample being positive for *B. burgdorferi* s.l. increased with estimated CC (GLM: $\beta = 7.82 \pm 0.38$, $p < 0.001$).

Infection prevalence for *A. phagocytophilum* was generally lower than for *B. burgdorferi* s.l. (Table S4.1). The highest prevalence of *A. phagocytophilum* was measured in forest, followed by open forest. NIP was lowest in open shrub (Figure 4b). Log-odds of a sample testing positive for *A. phagocytophilum* increased with CC, but the correlation was less pronounced than for *B. burgdorferi* s.l. (GLM: $\beta = 25.02 \pm 12.09$, $p = 0.039$).

Of all 320 *I. ricinus* nymphs positive for *B. burgdorferi* s.l., we could identify 174 samples to genotype. The most prominent genotype found was *Borrelia afzelii* (75%) followed by *B. spielmanni* (14%; Table S4.4). For *A. phagocytophilum*, 136 out of 162 positive samples were identified as ecotype I. Two samples were identified as ecotype II, and 24 samples could not be classified as either ecotype (Table S4.5). Pathogens detected in adult ticks are included in Table S4.6.

3.3.3 | Density of infected nymphs

Bracken had the highest density of nymphs infected with *B. burgdorferi* s.l. (Figure 4c), almost twice the density found in forest. Lowest DIN was measured in grassland, open shrub and open forest. Density of nymphs infected with *A. phagocytophilum* was highest in bracken and forest. DIN in the other habitat types was much lower.

For both pathogens, the DON had a stronger effect on DIN compared with NIP, since the variation in DON between habitat types

was higher (Figure 5). Measured DIN was much lower than the predicted RCC in open shrub and grassland, whereas it was much higher than RCC in bracken and forest habitats (Figure 4c,d). Thus, the contrasts between open and more densely vegetated habitat types were more pronounced than expected.

4 | DISCUSSION

L. agilis abundance in the study area strongly reduced estimated CC. Indeed, measured NIP was lowest in habitat types where *L. agilis* fed a large proportion of *I. ricinus* larvae. These findings suggest that *L. agilis* abundance plays a significant role in reducing the prevalence of both pathogens in questing nymphs. We found that overall, DON most strongly influenced DIN, which clearly shows the importance of abiotic conditions in determining tick-borne disease hazard.

4.1 | Mean tick burden and pathogens found in ticks collected from *Lacerta agilis*

Tick burden of *L. agilis* was considerably higher than previous estimates, indicating that *L. agilis* is indeed a relevant host for *I. ricinus* in the study area (Richter & Matuschka, 2006; Tjisse-Klasen et al., 2010). Mean tick burden was higher in individuals captured in habitat types where DON was also high, suggesting that *L. agilis* encounters more ticks where they are more abundant, in accordance with previous work on tick burdens of *P. major* (Heylen et al., 2013). Participation of adult *L. agilis* in reproductive behaviour may also lead to increased tick burden, as shown by Wiczorek et al. (2020). This may explain why males have a higher tick burden than females. During the mating season, males are generally more mobile, defending a territory and actively guarding females (Olsson, 1993). Additionally, higher testosterone levels may lead to compromised immune function, known as the immunocompetence-handicap (Hughes & Randolph, 2001; Olsson et al., 2000). As expected, only a fraction of the larvae feeding on *L. agilis* was infected with *B. burgdorferi* s.l. or *A. phagocytophilum*. While we cannot rule out that the lizard infected these larvae, it is more likely that the infection stems from previous (incomplete) feeding on an infected host or from co-feeding, which may occur sporadically (Voordouw, 2015).

4.2 | Nymphal density and the effects of weather and season

We found that the DON in the study area increased along the successional gradient, likely a direct result of microclimatic conditions. We suspect that host availability played a minor role in determining DON, due to the high abundance of fallow deer, which are important propagation hosts (Hofmeester et al., 2017). DON was by far highest in bracken. Due to the limited spatial extent of this habitat type in the study area, we were only able to sample three bracken sites. Nonetheless, we decided to include this habitat type because it is relevant to site management.

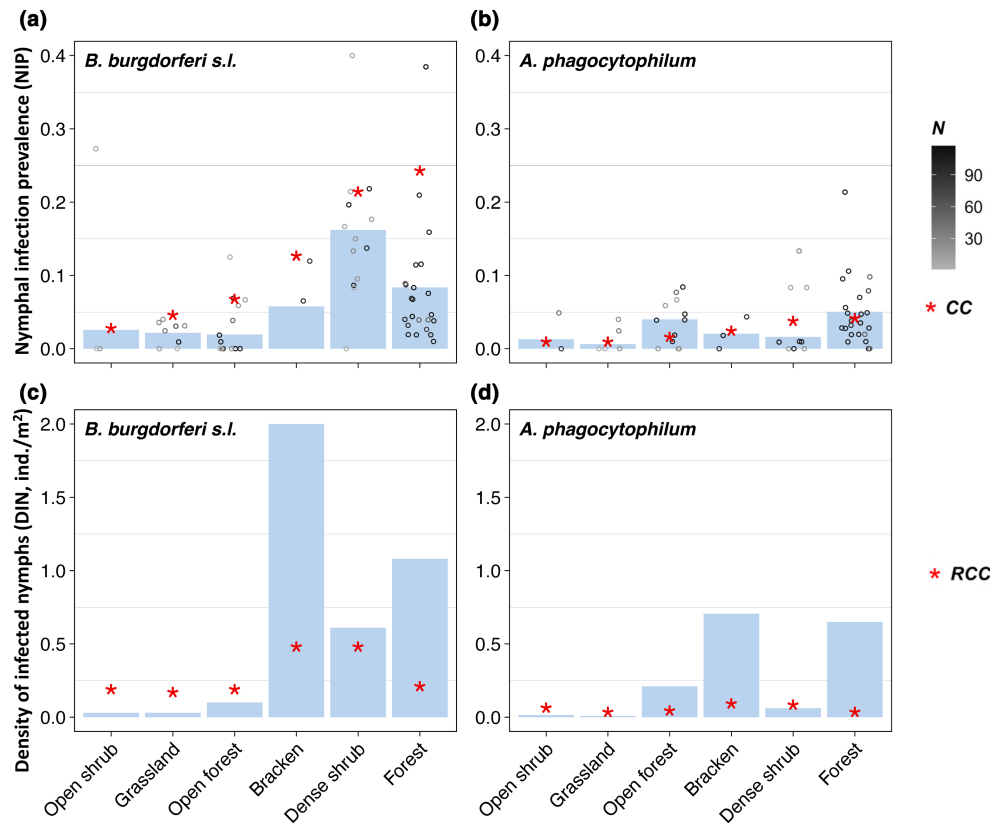


FIGURE 4 Upper bar plots showing nymphal infection prevalence of (a) *Borrelia burgdorferi s.l.* and (b) *Anaplasma phagocytophilum*. The values for community competence (CC) represent the result of our calculations. Points representing transects with a sample size lower than two were removed from the plot to improve readability ($n = 4$). Lower bar plots show measured density of infected nymphs for (c) *B. burgdorferi s.l.* and (d) *A. phagocytophilum*. The realized community competence (RCC) represents the expected density of infected larvae produced by the host community per 100 m².

The fern profits from atmospheric deposition of nitrogen, while being avoided by herbivores due to its toxicity (Ouden, 2000). However, vegetation structure may also have biased measured DON, as drag sampling success depends on the contact between cloth and vegetation (Tack et al., 2011), which may have led to an underestimation of DON in thorny *H. rhamnoides* shrub. Besides habitat type, weather conditions and season strongly influence tick survival and activity (Randolph & Storey, 1999). As expected, DON decreased from the start of the field season until the end (Gassner et al., 2011). Here, it must be noted that our data represent a snapshot in time, and differences between years could not be accounted for. In the Netherlands, 2021 was characterized by an unusually cold spring and wet summer, while the previous 3 years were exceptionally dry (KNMI, 2022b). Climate change scenarios predict irregular weather events to increase in frequency, underlining the necessity to conduct multi-season field studies to acquire comparable datasets (IPCC, 2022).

4.3 | NIP and the role of *Lacerta agilis* in the context of community competence

Our estimation of CC was largely determined by the relative densities of the four main host species. Other host species were of

comparatively little relevance, in agreement with the findings of Hofmeester et al., 2016. By deriving mean tick burden and infection prevalence of the host assemblage from literature, we accept the possibility that these estimated values differ in our study area due to local conditions that we did not account for. For example, it is likely that, as observed here in *L. agilis*, tick burdens of other host species is lower in habitat associated with lower DON (Heylen et al., 2013). This might explain why we consistently overestimate CC for both pathogens. Even with some variation in the actual contribution of host species to the CC, we expect our results to be robust (Table S2.1; Figure S2.1).

The fact that differences in NIP could be observed on a habitat level despite the high degree of spatial heterogeneity indicates that NIP is determined at a fine spatial resolution. CC of surrounding habitat had no effect on NIP, which leads us to conclude that spill-over from adjacent habitat patches is limited. A possible follow-up would be to measure the relative abundance and larval burden of relevant host species as well as NIP at a finer spatial resolution, so that also the indirect effects of spatial heterogeneity and the host community structure on DON and NIP can be estimated. In any case, the prevalence of both pathogens was lower in habitats where *L. agilis* fed the majority of *I. ricinus* larvae. This strongly suggests that *L. agilis* acted as the main dilution host for both *B. burgdorferi s.l.*

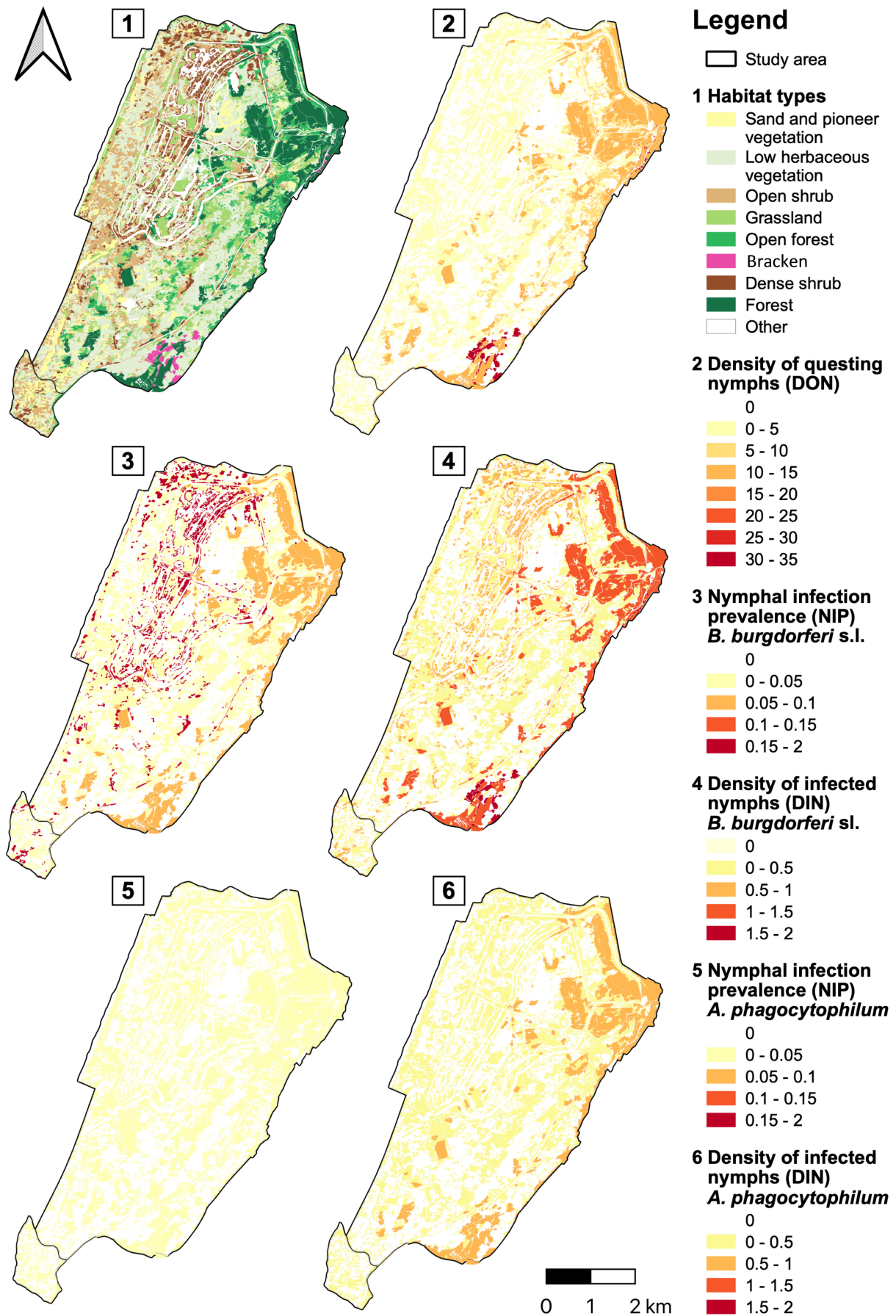


FIGURE 5 Maps showing the different habitat types in the study area (1), the mean density of questing nymphs (2), the nymphal infection prevalence (NIP) for *Borrelia burgdorferi* s.l. (3), NIP for *Anaplasma phagocytophilum* (4), the density of infected nymphs for *B. burgdorferi* s.l. (5) and density of infected nymphs for *A. phagocytophilum* (6).

and *A. phagocytophilum*. While *L. agilis* inhabits a wide range of open habitats, the coastal dune ecosystem may be uniquely suited to observe this effect in situ, as rodent densities are low in the early successional habitats where *L. agilis* is abundant. In other ecosystems, where rodents and *L. agilis* occur sympatrically, differences in NIP might be more difficult to detect.

In epidemiological research, it is often stated that high biodiversity reduces CC for pathogens that rely on a natural reservoir (Randolph & Dobson, 2012). However, most authors emphasize that species composition and relative abundances of specific species predict NIP more accurately (LoGiudice et al., 2008). Here, we found large variation in NIP in a relatively small host species community. NIP then depends on the competence of the most prominent host species. Our results present an example of how the dominance of an incompetent reservoir host can significantly lower NIP. Here, the increase in NIP followed the successional gradient, which can be explained by shifts in host species assemblage. In early successional habitat types, most *I. ricinus* larvae feed on an incompetent host (*L. agilis*), whereas in late successional habitat types, competent hosts like rodents feed most of the larvae. In the United States, Ginsberg et al. (2021) and Ostfeld and Keesing (2000) have shown that the overall increase in Lyme disease prevalence at higher latitudes may be based on the same mechanism of shifts in tick-host associations from incompetent host-dominated to competent host-dominated habitat. After all, the successional gradient in coastal dunes is not unlike the latitudinal gradient in climate and vegetation.

4.4 | Density of infected ticks and implications for hazard

Although our study highlights an important role for *L. agilis* as dilution host, our findings also indicate that DON is a more important factor than NIP in determining DIN. Given that DON is largely determined by abiotic conditions and habitat structure, this implies that the off-host environment has a larger impact on tick-borne disease hazard than the reservoir competence of the host community (Gandy et al., 2021). The differences between expected DIN (i.e. RCC) and observed DIN clearly illustrate this. In habitat types suitable for *I. ricinus* (forest, bracken), measured DIN far exceeded RCC, as infected hosts continuously produce infected nymphs, and abiotic conditions are optimal for tick survival and activity. In habitat types unsuitable for *I. ricinus* (open shrub, grassland), the relationship was reversed, and measured DIN was much lower than the host community could theoretically produce, most likely because survival and activity of *I. ricinus* are limited.

5 | CONCLUSION

The current study shows how habitat heterogeneity shapes the local host species assemblage and thereby the CC for tick-borne diseases.

Incompetent hosts (*L. agilis*) are most prominent in early successional stages but are replaced by competent hosts (rodents) in late successional stages, resulting in an increase in infection prevalence along the successional gradient. Thus, *L. agilis* acts as a dilution host in early successional habitat. However, tick density plays an even more important role than infection prevalence in determining disease hazard. Early successional habitat is characterized by low tick density, as microclimatic conditions are unfavourable for *I. ricinus*. The conservation and restoration of nutrient-poor habitat types in coastal dunes and similar ecosystems can, therefore, lower human infection hazard for tick-borne pathogens both directly (through lowering nymphal density) and indirectly (through lowering CC, resulting in lower infection prevalence). In the present study, the highest DIN was measured in bracken, making this the most relevant habitat type for hazard reduction. As bracken is invasive in Dutch coastal dunes, active vegetation management by mowing and removal is an option for reducing hazard to the public. Additionally, closing or constructing walking paths in- or outside of hazardous areas may reduce risk of tick bites for humans. The hazard maps created in the scope of this study are an example of how the results of ecological research may serve as a tool for the management of natural areas.

AUTHOR CONTRIBUTIONS

Annemarieke Spitzen-van der Sluijs, Willem Frederik de Boer, Helen Esser and Hein Sprong conceived the ideas and set up the framework; Annemarieke Spitzen-van der Sluijs and Hein Sprong supervised the project and acquired the funding; Vincent van der Spek, Willem Frederik de Boer and Hein Sprong provided the resources and materials for fieldwork and analysis; Manoj Fonville, Helen Esser, Willem Frederik de Boer and Clara Köhler designed the methodology; Clara Florentine Köhler collected the data; Manoj Fonville and Clara Florentine Köhler analysed the data; Clara Florentine Köhler led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available from Dryad Digital Repository <https://doi.org/10.5061/dryad.zpc866tcq> (Köhler et al., 2023).

ORCID

Clara Florentine Köhler  <https://orcid.org/0000-0001-5808-9640>

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