The impacts of host community composition on Lyme disease risk in Scotland

by

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Abstract

Emergence of zoonoses are driven by multiple factors, ranging from climate change to urbanization and human behaviours. Because many zoonotic pathogens are maintained in wild reservoir hosts, these factors of emergence may affect disease risk by changing host community parameters. Thus, it is important to understand the effect of host community composition on disease risk. This is particularly relevant for vector-borne zoonoses as host community composition might affect both reservoir host and vector populations. In the northern hemisphere, Lyme disease, a zoonosis caused by the bacterial complex *Borrelia burgdorferi* sensu lato, is the most prevalent vector-borne disease affecting humans. Transmitted by Ixodid ticks, its epidemiological cycle is complex and depends on environmental factors and host community composition, which together, influence both tick survival and the prevalence of *B. burgdorferi* s.l. Small mammals are competent reservoir hosts for *B. afzelii*, a genospecies belonging to the *B. burgdorferi* s.l. complex, while birds can transmit *B. valaisiana* and *B. garinii*, two other genospecies in the complex. Thus, pathogen prevalence will depend on the abundance of competent reservoir hosts in an environment. Deer species, on the other hand, are non-competent hosts and could, theoretically, lower *B. burgdorferi* s.l. prevalence by diverting immature ticks from feeding on competent reservoir hosts, a concept called the dilution effect. However, deer also act as the main tick reproduction hosts, feeding adult female ticks, and can therefore maintain high tick populations. In this thesis, I used a range of approaches (large-scale cross-sectional survey, deer exclosure and wood mouse supplementary feeding experiment) to test hypotheses and understand how host community composition drives Lyme disease hazard (defined here as the density of infected nymphal ticks) in Scotland and to investigate the drivers of host communities.

I investigated how the ratio of small mammals to deer affected Lyme disease hazard by conducting a large cross-sectional survey across sites selected to specifically cover the full range of deer densities (Chapter 2) and collecting data on host abundance (deer and small mammals), tick density and pathogen prevalence. There was a positive association between the density of infected

nymphs (DIN) for *B. afzelii* and deer density, regardless of small mammal abundance. I also observed a positive association between deer density and questing nymph density and a strong dilution effect by roe deer on *B. afzelii* prevalence. These results suggest that the role of deer in driving tick density can outweigh their role in diluting *B. burgdorferi* s.l. infection and thus, deer, rather than small mammals drove Lyme disease hazard in this system. When investigating the factors influencing host communities, I found evidence for interspecific interactions between roe and red deer and between deer and small mammals. Roe deer density was also negatively associated with human activity, which therefore has the potential to impact Lyme disease hazard.

Having shown how the full range of deer densities shapes Lyme disease hazard, which also suggested that deer may affect small mammal densities (Chapter 2), I then wanted to investigate further the possibility that high deer densities may affect Lyme disease by causing ecological cascades, through an impact on vegetation and small mammals (Chapter 3). I used an experimental design consisting of replicated fenced deer exclosures to investigate the effects of high deer density versus deer absence on Lyme disease hazard through ecological cascades. Consistent with my predictions, high deer density plots had 18 times more questing nymphs compared to plots where deer were absent. High deer density plots also had 13 times fewer small mammals and were associated with shorter and sparser vegetation and shorter trees, highlighting the impacts of browsing pressure by deer on small mammal communities. I found that the reduction in competent host abundance had repercussions on *B. burgdorferi* s.l. prevalence in questing ticks, which was twice as high in deer exclusion plots (2.2% in deer exclusion plots vs 1.0% in high deer density plots). Despite the negative effect of high deer density on *B. burgdorferi* s.l. prevalence, DIN was five times higher in high deer density plots. These results demonstrate that the positive effect of deer on tick density can outweigh their negative effect on *B. burgdorferi* s.l. prevalence caused by a dilution effect and through their negative effects on small mammals.

In chapters 2 and 3, I found that deer appeared to be more important than small mammals in driving DIN, through their strong role as tick reproduction hosts, irrespective of any dilution effects on prevalence. However, few of my sites had

high small mammal densities while many had high deer densities. I therefore wanted to test the effect of a variation in small mammal abundance on ticks, *B. burgdorferi* s.l. prevalence and DIN using a supplementary feeding experiment to increase small mammal densities. More specifically, the objective for this study (Chapter 4) was to understand the effects of a change in food resources on small mammal populations and the repercussions on Lyme disease hazard. Fluctuations in resources availability are common occurrences in nature so it is important to understand the consequences this could have for disease hazard. This involved an experimental design where two trapping grids out of four were supplemented with food for two consecutive years. Density of live-trapped wood mice (*Apodemus sylvaticus*) was twice as high in food supplemented grids during the year of treatment. Food supplementation *per se*, which reflects what would happen after a mast seeding event, did not affect the density of nymphs, *B. burgdorferi* s.l. prevalence or DIN. However, there was a positive correlation between wood mouse abundance and questing nymph abundance the following spring. I hypothesised a negative association between wood mouse abundance and the prevalence of the bird associated *B. garinii* and *B. valaisiana* (dilution effect where more ticks would be feeding on mice instead of birds) but I did not find evidence for that. Unexpectedly, there was a positive association between wood mouse abundance and DIN for *B. garinii* and *B. valaisiana* the following spring. This could occur either if mice acted like deer did in Chapters 2 and 3, as a tick amplification host, since they increased nymph density but not *B. garinii* and *B. valaisiana* prevalence and/or if the food supplementation that increased mice also increased birds, which I did not measure. Even though food supplementation *per se* did not have a direct impact on DIN, wood mouse abundance had a positive effect on tick density and DIN for *B. garinii* and *B. valaisiana* and these results highlight how variations in food resources can affect small mammals and Lyme disease hazard.

The cross-sectional study (Chapter 2) is the first, to my knowledge, to test how the ratio of competent reservoirs hosts (small mammals) to non-competent hosts (deer) affects Lyme disease hazard, whilst also specifically selecting sites with the full range of deer densities to robustly test the dilution effect. While I predicted that DIN should be modulated by variations in the density of both hosts, my results really pointed to deer density being the key component driving Lyme disease

hazard. This result, which was further confirmed in Chapter 3, could be used for deer management decisions in Scottish woodlands. This thesis also demonstrates the complexity of Lyme disease ecology: not only due to the multiple host types and multiple pathogens in the *burgdorferi* s.l. complex, but also in terms of how the hosts themselves interact and affect each other's distributions and densities. There is now clearly a need for further research to better understand the mechanisms driving Lyme disease hazard, including interspecific interactions and other drivers suggested in this thesis, such as human disturbance and food resources, that affect host abundance. Combining experimental designs and large-scale surveys allowed a better understanding of the effects of deer and small mammals simultaneously on Lyme disease. Indeed, the experimental designs (Chapters 3 and 4) offered the chance to vary the density of one host species and observe the cascading effects on other hosts, tick density and *burgdorferi* s.l. prevalence. Results from the cross sectional-survey (Chapter 2) not only corroborated the key associations observed but, importantly, allowed me to robustly test the dilution effect by examining the effects of a wide range of deer densities in the natural environment on DIN. The results obtained can be used to understand and predict how environmental changes (e.g. increase of resources, increase of human activity) might impact host species and Lyme disease hazard. Furthermore, they could be used to predict the effects that a change in deer management might have on tick density and DIN.

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Author's Declaration

I declare that the work in this thesis is mine unless otherwise stated. No part has been submitted for another degree. The material has been prepared in collaboration with co-authors as follow:

Chapter 2: In preparation for submission as: Gandy S. L, Millins C. L, Kilbride E, Gilbert L, Biek R. The impacts of host community composition on Lyme disease hazard: a cross-sectional survey in Northeast Scotland.

Study design was developed by SG, CM, LG and RB. Fieldwork was carried out by SG. EK optimized the qPCR protocol and laboratory testing was carried out by SG. Statistical analyses were performed by SG with input from Dr Paul Johnson. Manuscript was written by SG with comments from CM, LG and RB.

Chapter 3: In preparation for submission as: Gandy S. L, Millins C. L, Kilbride E, Biek R, Gilbert L. Experimental evidence for cascading effects of deer on Lyme disease pathogen, ticks and their rodent reservoirs.

Study design was developed by SG, CM, LG and RB. Fieldwork was carried out by LG in 2013 and 2014 and by SG in 2017, 2018 and 2019. EK optimized the qPCR protocol and laboratory testing was carried out by SG. Statistical analyses were performed by SG. Manuscript was written by SG with comments from CM, LG and RB.

Chapter 4: Gandy S. L, Hall J, Kilbride E, Sweeny A, Venkatesan S, Babayan S, Pedersen A, Millins C. L, Gilbert L, Biek R. Unexpected consequences of experimental evidence for the effect of food supplementation on Lyme disease hazard through a change in competent wood mouse density

Study design was developed by SG, CM, LG and RB. Experimental design was set up by AP, AS and SB. JH, AS, SV, SB and AP conducted rodent trapping fieldwork in 2017 and 2018 and SG collected tick samples in 2018 and 2019. Laboratory testing was carried out by SG and EK optimized the qPCR protocol. Statistical

analyses were performed by SG. Manuscript was written by SG with comments from CM, LG and RB.

Collaborators.

Dr Lucy Gilbert, Dr Roman Biek and Dr Caroline Millins are my PhD supervisors. Dr Lucy Gilbert shared data used in Chapter 3 that she collected in 2013 and 2014. Elizabeth Kilbride optimized the qPCR protocol that was used to analyse samples collected for Chapters 2, 3 and 4. Dr Amy Pedersen, Dr Simon Babayan, Dr Amy Sweeny, Dr Jessica Hall and Saudamini Venkatesan conducted rodent trapping fieldwork (Chapter 4) and shared rodent data used in Chapter 4. Dr Paul Johnson provided statistical help for Chapter 2.

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Abbreviations

AICc: Corrected Akaike information criteria

CI: Confidence interval

df: degrees of freedom

DIN: Density of infected nymphs

DNA: Deoxyribonucleic acid

dNTP: deoxynucleotide triphosphates

DON: Density of nymphs

EDTA: Ethylenediaminetetraacetic acid

GLM: Generalize linear model

GLMM: Generalized mixed effect model

$MgCl_2$: Magnesium chloride

NIP: Nymphal infection prevalence

NH_4OH: Ammonium hydroxide

Osp : Outer surface protein

PCR: Polymerase chain reaction

qPCR: Quantitative polymerase chain reaction

SD: Standard deviation

s.l.: sensu lato

s.s.: sensu stricto

VIF: Variance inflation factor

1. Introduction

1.1. Emerging infectious diseases

"It was a cruel and horrible thing […]
None could be found to bury the dead […]
And they died by the hundreds, both day and night,
and all were thrown in those ditches and covered with earth."
Agnolo di Tura (ca 1350) – Recounting of the black death

For millennia, emerging infectious disease outbreaks have shaped humanity. From the black death wiping out a third of Europe in the 14th century (Morens *et al.*, 2008) to the 1918 influenza pandemic, in which the death toll was estimated to 50 million (Morens *et al.*, 2008; Taubenberger & Morens, 2006), and the ongoing AIDS (acquired immune deficiency syndrome) epidemic, having killed 32 million since the 1980's (WHO, 2018) and still taking more than 700,000 lives every year (WHO, 2018) those outbreaks had a role in shaping the world we live in today.

Even though a lot of progress has been made in recent centuries to reduce the toll of infectious diseases, such as the creation of rabies vaccine by Pasteur and Roux in 1885 or the discovery of penicillin in 1928 (Morens *et al.*, 2004), only two diseases have been successfully eradicated (smallpox in 1980 and rinderpest in 2001) and some forgotten pathogens in the western world still thrive in part of the developing world. Nowadays, re-emergence of known pathogens and emergence of new ones still account for 15 million of annual deaths and understanding the drivers are important (Table 1.1) (Morens *et al.*, 2004).

Table 1.1: Non exhaustive list of emerging infectious disease outbreaks in the recent past, the location of the emergence and the number of cases reported.

Disease	Location of emergence	Period	Human cases	Source
Zika	Americas Caribbean	2015-?	>850,000	Anderson *et al.*, 2016; Vorou, 2016; PAHO, 2018
SARS	Asia	2003	800 deaths	Graham *et al.*, 2013
AIDS	Worldwide	1981-?	>32M deaths	Morens *et al.*, 2004
Ebola	West Africa	1976-?	4,572 deaths as of 2015	Muyembe-Tamfum *et al.*, 2012; Alexander *et al.*, 2015
Plague	China	1986-2005	507	Gupta & Ballani, 2014; Shi *et al.*, 2018; WHO, 2010
	DRC	2004-2009	7,811	
	Madagascar	2004-2009	3,454	
H5N1	Hong Kong	1997-1998	18	Bui *et al.*, 2016; WHO, 2020
	South Asia	2003-2012	399	
Covid19	China	2020-?	>7M	

Amongst emerging infectious diseases, 75% are zoonoses (Rhyan & Spraker, 2010), transmitted to humans from animals after a spill over event (Daszak *et al.*, 2000; Engering *et al.*, 2013). Vector-borne diseases, relying upon vectors, living organisms that carry and transmit pathogens from one host to another (Gratz, 1999; Gubler, 2009) account for 17% of all infections (WHO, 2020) (Table 1.2).

Table 1.2: Non exhaustive list of the annual number of cases and death toll for vector-borne diseases worldwide in 2014. Source: WHO, 2014.

Disease	Annual number of cases	Annual death toll	Vector(s) involved
Malaria	207M	627,000	*Anopheles* Mosquitoes
Dengue	2.4M cases in 2010	2.5%	*Aedes* mosquitoes
Yellow fever	200,000	30,000	*Aedes* and *Haemagogus* mosquitoes
Japanese encephalitis	50,000	10,000	*Culex* mosquitoes
Leishmaniasis	1.3M	~25,000	Sand-flies
Crimea Congo fever	10,000-15,000	40%	*Hyalomma* ticks
Schistosomiasis	249M treated in 2012		Snails (intermediate hosts)

In addition to the lives lost due to vector-borne diseases, they represent an important economic burden. For instance the cost of dengue was 8-9 billion USD in 2013 (Shepard *et al.*, 2016) while the drug to treat leishmaniasis costs between 30 and 1,500 USD per patient (Okwor & Uzonna, 2016). To reduce the risk of vector-borne diseases, understanding the factors affecting vector populations and pathogen transmission is necessary.

Vector-borne diseases have complex life cycles depending on both vector and host populations and thus, what affects them (Figure 1.1). Factors of emergence are diverse and include environmental changes expanding host and vector distribution ranges (Githeko *et al.*, 2000), sexual practices (Hastings & Fikrig, 2017) and increased contact rate between humans, reservoir hosts and vectors due to urbanization and habitat fragmentation (Parrish *et al.*, 2008; Schrag & Wiener, 1995). For instance, urban areas can create suitable habitats for vectors, thus increasing their density and contact rates with humans (Gibbs *et al.*, 2006; Mackenzie *et al.*, 2004).

Changes in vector density and infection rates in vector populations will thus affect disease hazard, which is defined by the density of infected vectors, and depends on the abundance of competent reservoir hosts (Allan *et al.*, 2003; Kilpatrick *et al.*, 2017). Their presence and abundance can be affected by climate change (Caminade *et al.*, 2019), anthropogenic pressure and interspecific competition (Hoyer *et al.*, 2017; Mkoji *et al.*, 1999) (Figure 1.1). For example, a study conducted in Kenya showed that the introduction of crayfish in lakes resulted in a decrease in schistosomiasis infections in children by reducing the abundance of intermediate snail hosts (*Bulinus africanus*) through predation and competition (Mkoji *et al.*, 1999).

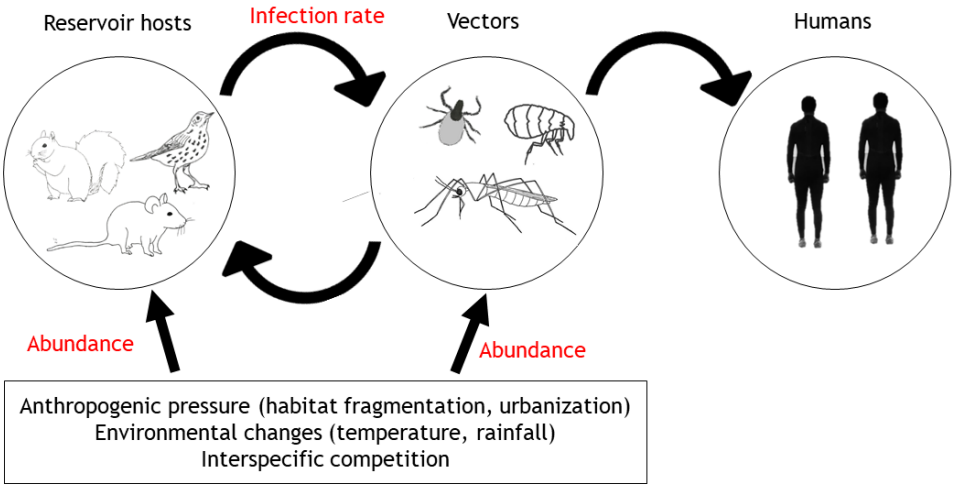


Figure 1.1: Conceptual figure illustrating the simplified epidemiological cycle of vector-borne diseases. Reservoir host abundance is determined by anthropogenic pressure, environmental changes and interspecific competition and affects infection rates in vectors. Vector abundance is influenced by the same biotic factors and can also be influenced by host abundance in the case of tick-borne diseases. Disease hazard for humans is defined by the density of infected vectors. For some vector-borne diseases, vectors can also be infected while taking a blood meal from an infected human.

In North America and Europe, Lyme disease, a bacterial disease transmitted by Ixodid ticks is the most prevalent vector-borne zoonosis (Steere *et al.*, 2004; Walter *et al.*, 2017). Over the past few decades, its geographical range has rapidly expanded and its incidence has risen. Therefore, Lyme disease will be the focus of this thesis.

1.2. Lyme disease as a study system - history and incidence rates

Lyme disease was first identified in 1977 in Old Lyme, Connecticut (US) (Steere *et al.*, 1977) and the spirochetes causing the disease were first isolated in humans in 1981 and in Ixodid ticks in 1985 (Barbour & Hayes, 1986; Burgdorfer *et al.*, 1985) (Figure 1.2). After becoming a notifiable disease in the US in 1991, a vaccine was approved but it was withdrawn from the market three years later due to poor market performance (Nigrovic & Thompson, 2007). Even though considered as a recent emergence, researchers have found the pathogen responsible for Lyme disease in "Otzi", a man who lived 5,300 years ago and was discovered preserved in a glacier on the border between Italy and Austria, showing that it has been circulating for much longer than originally thought (Kean *et al.*, 2013) (Figure 1.2).

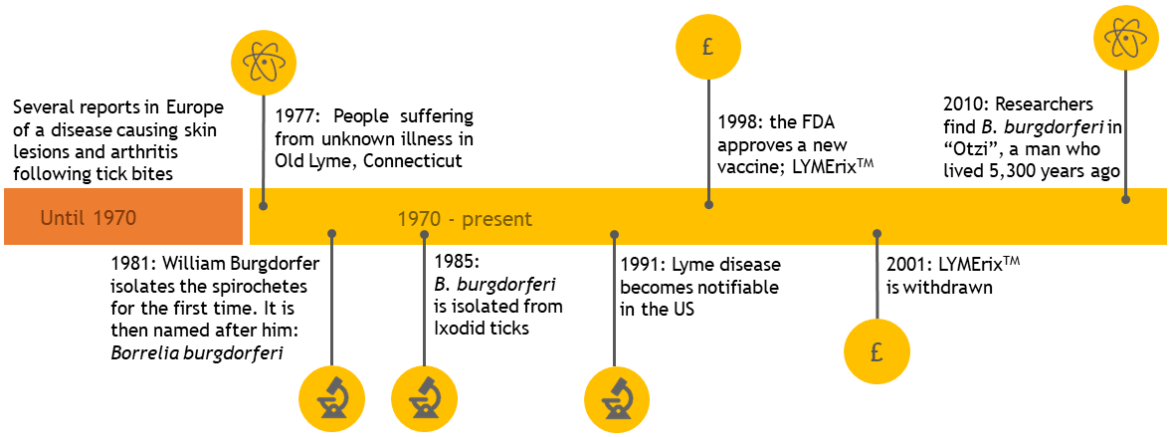


Figure 1.2: Conceptual figure showing the timeline illustrating the history of Lyme disease. The microscope symbol represents major stepping stones in Lyme disease research, the £ symbol represents events of economic importance and the atom symbol discovery in the history of Lyme disease.

Lyme disease has been emerging in both Europe and North America over the past few decades (Table 1.3). For instance in Scotland, the region of the United Kingdom having the highest incidence rate (Scotland: 37.3/100,000, rest of UK: 10.24/100,000), the number of cases increased from 1.6/100,000 in 2001 to 12.1/100,000 in 2012, a 7-fold increase over 11 years (Cairns *et al.*, 2019). More recent data for Scotland are available but not comparable as Lyme disease stopped being a notifiable disease in 2010 (Mavin *et al.*, 2015).

Table 1.3: Non exhaustive list of the increase in incidence rate of Lyme disease per 100,000 in European countries and North America in recent years based on surveillance data.

Country	Period	Increase in incidence rates (/100,000)	Source
Europe			
Finland	1995-2014	7 (1995) to 21 (2014)	Sajanti *et al.*, 2017
France	2009-2018	46 (2009) to 104 (2019)	Santé publique, 2019
Hungary	1998-2010	11.2 (1998) to 23 (2010)	Trájer *et al.*, 2013
Netherlands	1994-2007	39 (1994) to 134 (2007)	Hofhuis *et al.*, 2015
Scotland	2001-2012	1.6 (2001) to 12.1 (2012)	Cairns *et al.*, 2019
Slovakia	1999-2006	10.5 (1999) to 18.3 (2006)	Svihrova *et al.*, 2011
North America			
Canada	2009-2015	0.4 (2009) to 2.6 (2015)	Gasmi *et al.*, 2017
Pennsylvania (US)	2008-2017	32.1 (2008) to 92.9 (2017)	Pennsylvania department of health, 2019
Wisconsin (US)	1990-2018	10 (1990) to 50 (2018)	Wisconsin department of health, 2020

1.3. The pathogen causing Lyme disease – *Borrelia burgdorferi* s.l.

Lyme disease is caused by a gram-negative spirochete bacteria belonging to the complex *Borrelia burgdorferi* sensu lato and it is transmitted by hard bodied ticks (Acari: Ixodidae) (Barbour & Hayes, 1986; Johnson *et al.*, 1984). Within this complex, 21 different genospecies have been identified so far (Table 1.4).

Table 1.4: List of the genospecies belonging to the complex *Borrelia burgdorferi* sensu lato identified since 1990. Genospecies in bold are known to be pathogenic to humans. If a * follows, it signifies that it rarely infects humans and ** means that the pathogenicity remains unclear. Source: Casjens *et al.*, 2011; Ivanova *et al.*, 2014; Margos *et al.*, 2011; Pritt *et al.*, 2016; Stanek & Reiter, 2011; Veinović *et al.*, 2016.

Year	Genospecie	Location	Genospecie	Location
1990s	***B. garinii***	Europe/Asia	***B. burgdorferi* s.s.**	Europe/North America
	B. afzelii	Europe/Asia	*B. andersonii*	North America
	B. japonica	Asia	*B. tanukii*	Asia
	B. turdi	Asia	***B. valaisiana*****	Europe/Asia
	B. lusitaniae*	Europe	***B. bissettii****	Europe/North America
2000s	*B. sinica*	Asia	***B. spielmani***	Europe
	B. Yangtze	Asia	*B. californiensis*	North America
	B. Americana	North America	***B. bavariensis***	Europe
	B. kurtenbachii	North America	*B. carolinensis*	North America
2010s	*B. chilensis*	South America	*B. mayonii*	North America
	B. finlandensis	Europe		

In Europe, six genospecies (*B. burgdorferi* s.s., *B. afzelii*, *B. garinii*, *B. bissettii*, *B. bavariensis* and *B. spielmani*) can cause the disease in humans while the pathogenicity of *B. lusitaniae* and *B. valaisiana* remains unclear (Kurtenbach *et al.*, 2006; Richter *et al.*, 2004; Stanek *et al.*, 2012). *B. afzelii* and *B. garinii* are generally the most common genospecies found in Europe followed by *B. burgdorferi* s.s., *B. valaisiana* and *B. lusitaniae* (Mannelli *et al.*, 2012; Rauter & Hartung, 2005; Strnad *et al.*, 2017).

In Europe, several vertebrate species including rodents, shrews (*Sorex* spp.) and birds are competent reservoir hosts for *B. burgdorferi* s.l., meaning that they can carry and maintain the pathogen and serve as a source of infection. Reservoir competency for B. burgdorferi s.l. is usually assessed using xenodiagnosis where noninfected larvae are permitted to feed on infected hosts. A host will be considered competent if newly moulted nymphs are infected with the pathogen,

as they would have acquired the infection from that host (Kurtenbach et al., 1994; Tälleklint and Jaenson, 1994). Although some studies also tested engorged ticks and host blood or tissue sample to detect the pathogen (Dubska et al., 2009; Hanincová et al., 2003a), a positive result does not necessarily confirm that the host can transmit the pathogen to the tick as co-feeding could have occurred. In the case of *B. burgdorferi* s.l., there are associations between host species and the different genospecies (Kurtenbach *et al.*, 1998b) (Table 1.5).

Table 1.5: Non exhaustive list of competent reservoir hosts for *Borrelia burgdorferi* sensu lato in European hosts and associated genospecies. *B.b.* s.s. stands for *B. burgdorferi* s.s.

Host	Associated genospecies	Sources
Wood mouse (*Apodemus sylvaticus*)	*B. afzelii* *B. b.* s.s.	Kurtenbach *et al.*, 1994[3], 1998[2], 2002[2]; Tälleklint and Jaenson, 1994[3]; Humair *et al.*, 1999[3]; Hanincová *et al.*, 2003a[1,4]
Bank vole (*Myodes glareolus*)	*B. afzelii* *B. b.* s.s.	Kurtenbach *et al.*, 1994[3], 2002[2]; Tälleklint and Jaenson, 1994[3]; Humair *et al.*, 1999[3]; Hanincova *et al.*, 2003a[1,4]
Field vole (*Microtus agrestis*)	NA	Tälleklint and Jaenson, 1994[3]; Humair *et al.*, 1999[3]
Shrew (*Sorex* spp.*)*	NA	Tälleklint and Jaenson, 1994[3]; Brisson and Dykhuizen, 2004[1]
Grey squirrel (*Sciurus carolinensis*)	*B. afzelii* *B. b.* s.s. *B. garinii*	Kurtenbach *et al.*, 2002[2]; Brisson and Dykhuizen, 2004[1]; Salkeld *et al.*, 2008[4]
Black bird (*Turdus merula*)	*B. garinii* *B. valaisiana*	Kurtenbach *et al.*, 2002[2]; Dubska *et al.*, 2009[1]
Song thrush (*Turdus philomelos*)	*B. garinii* *B. valaisiana*	Dubska *et al.*, 2009[1]; James *et al.*, 2011[1]
Great tit (*Parus major*)	*B. garinii* *B. valaisiana*	Dubska *et al.*, 2009[1]; James *et al.*, 2011[1]
Lizard	*B. garinii* *B. valaisiana*	Kurtenbach *et al.*, 2002[2]

Reservoir competency obtained from: 1: engorged ticks, 2: cell culture in presence of serum, 3: xenodiagnosis, 4: host tissue/blood

The most common symptoms of Lyme disease may include a characteristic bull's eye rash, fatigue and flu-like symptoms (Anguita *et al.*, 2003). If left untreated, secondary disease might include skin rashes, acute arthritis (10-20% of patients), meningitis (10%) and carditis (8%) (Anguita *et al.*, 2003). There are associations between clinical presentations and different genospecies. For instance, rashes are more prevalent if the infection is caused by *B. afzelii* while neurologic manifestations are more common with *B. garinii* and arthritis with *B. burgdorferi*

s.s. (Ryffel *et al.*, 1999; Stanek *et al.*, 2012; van Duijvendijk *et al.*, 2015; Vollmer *et al.*, 2013) and understanding the drivers of different genospecies is clinically relevant.

1.4. The vector – Ixodid ticks

1.4.1. The ecology of Ixodid ticks

Ixodid ticks are ectoparasites characterized by their scutum, a hard dorsal plate distinguishing them from the Argasidae family, and comprise about 650 species. Within Ixodid ticks, ticks from the genus *Ixodes* are carriers of the Lyme disease pathogen *B. burgdorferi* s.l. (Swanson *et al.*, 2006) as well as other pathogens causing diseases in humans and livestock (e.g. for humans: anaplasmosis, babesiosis, Powassan disease, tick-borne encephalitis. For livestock: babesiosis, louping ill, theileriosis. Source: CDC, 2020).

Ixodid ticks are generalists (Hofmeester *et al.*, 2016), feeding on a wide range of vertebrates, and alternate between periods on the hosts while taking a blood meal and off the host when moulting to their next life-stage (Oliver, 1989). They are exophilic and, unlike other species that live inside nests, Ixodid ticks actively seek their hosts. Ticks go through three life-stages; larvae, nymphs and adults and subadult stages require a blood meal to moult and develop to the next life-stage. After hatching, larvae find their first host and feed for 3-4 days (Oliver, 1989). While transovarial transmission has not been demonstrated for *B. burgdorferi* s.l. (Richter *et al.*, 2012; Rollend *et al.*, 2013), a study conducted in the Netherlands found that 0.6% of questing larvae were infected with *B. afzelii* (van Duijvendijk *et al.*, 2016a). While these findings imply that a low proportion of larvae could be infected, mechanisms for that are unknown (van Duijvendijk *et al.*, 2016a). After moulting into nymphs, they feed on a second host for 4-8 days and can infect naïve hosts or humans if they carry a pathogen from their previous blood meal (Oliver, 1989). They then moult into adults and female ticks will take a last blood meal for 7-12 days on large size vertebrates before mating and laying eggs. Adult males do not gorge on blood and will remain on hosts until they mate (Oliver, 1989) (Figure 1.3).

Ixodid ticks generally complete their life cycle in three years. However, they can enter a quiescence stage, called behavioural diapause, if environmental conditions do not allow their survival (e.g. no hosts available, drop in temperatures, drought) and suppress host seeking activity (Belozerov, 1982). This period of quiescence can delay the completion of their life cycle and could therefore reduce their reproductive fitness (i.e. their ability to successfully reproduce and pass on their genes) (Gray *et al.*, 2016).

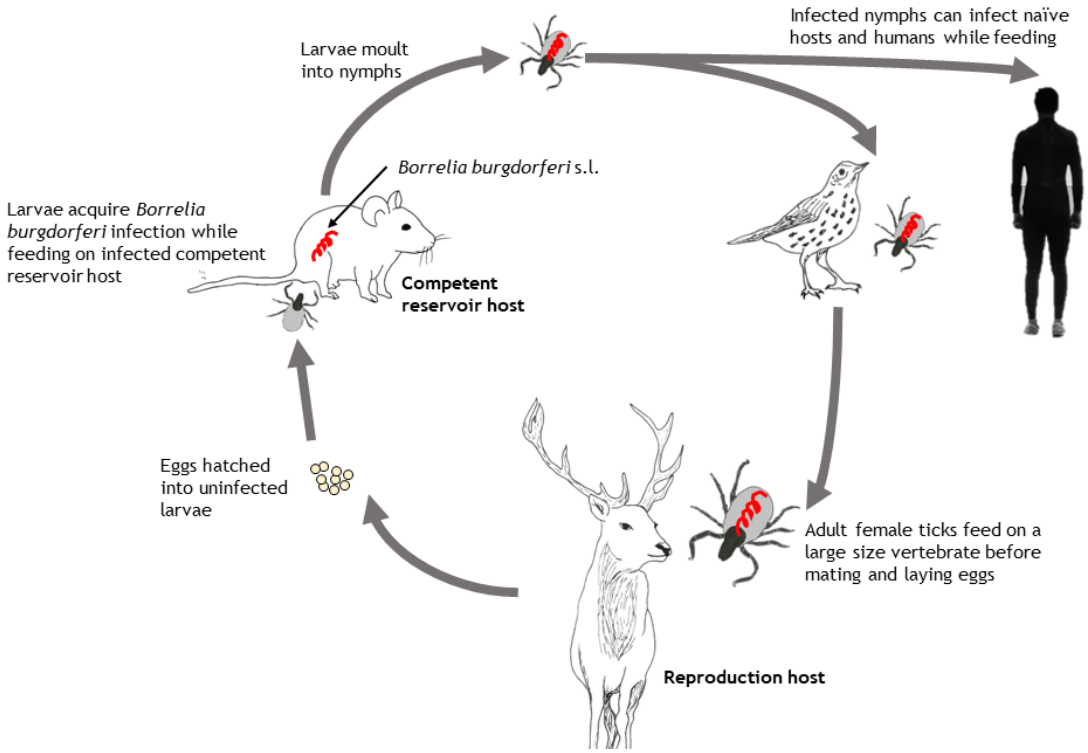


Figure 1.3: Conceptual figure illustrating the life cycle of Ixodid ticks and *B. burgdorferi* s.l. transmission. Uninfected larvae take a blood meal on a first host and can potentially get infected while feeding. They moult into nymphs and can infect naïve hosts and humans while taking their second blood meal. After moulting into adults, adult females must feed on a large size vertebrate, called a reproduction before mating and laying eggs. Source: adapted from Brisson *et al.*, 2012.

In North America, two species of ticks are known vectors for Lyme disease; *Ixodes pacificus* in Western US and *I. scapularis* in Eastern US while *Ixodes ricinus* transmits the pathogen in Europe and *Ixodes persulcatus* in Asia (Figure 1.4) (Oliver, 1996; Stanek *et al.*, 2012).

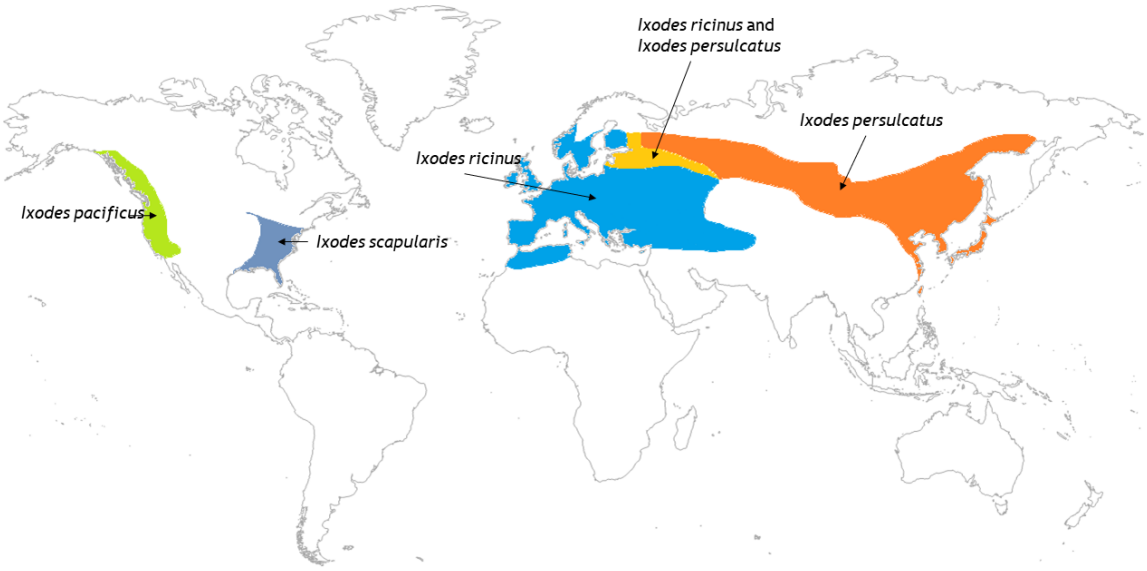


Figure 1.4: World map showing the distribution ranges of the vectors of Lyme disease (Ixodes species) in North America, Europe, North Africa and Asia. Source: Adapted from Stanek *et al.*, 2012.

Ixodid ticks can use vibration and carbon dioxide as well as heat to detect hosts passing by and use these cues to attach to them (Anderson *et al.*, 1998; Oorebeek *et al.*, 2009; Vassallo & Pérez-eid, 2002). Several studies have tried to implement blood meal analysis methods to identify which hosts questing nymphs have fed on in their previous life-stage and results in Europe showed ungulates (24-38% of blood meals), rodents (14-25%) and birds (12-48%) to be important hosts for larval ticks (Figure 1.5) (Humair *et al.*, 2007; Pichon *et al.*, 2005). Mustelids and foxes also represent a proportion of detected blood meals in these studies (10% and 5% respectively). However, as a broad array of vertebrate species must be detected, methods are highly susceptible to contamination and, due to the lag between host feeding and blood meal analysis of questing nymphs, sensitivity is usually less than 50% of ticks tested (Estrada-Peña *et al.*, 2013; Humair *et al.*, 2007; Pichon *et al.*, 2005).

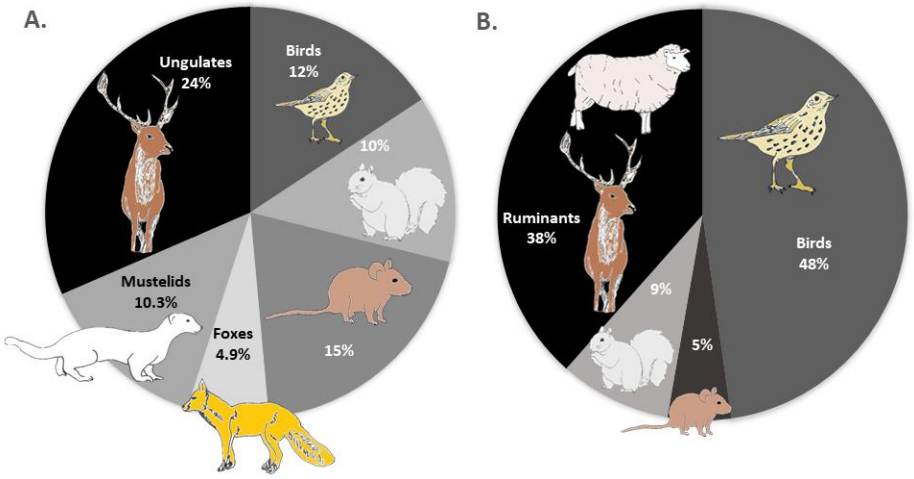


Figure 1.5: Pie charts representing the proportion of various mammal species or group of species as part of Ixodid nymphal ticks bloodmeal in Europe. Due to the lag between larvae feeding and questing nymphs being analysed, sensitivity is less than 50% of ticks tested. Sources: Adapted from A- Humair *et al.*, 2007; B- Pichon *et al.*, 2005 based on data from Europe.

1.4.2. *Ixodes ricinus* burden on hosts

Ixodes ricinus ticks must find a suitable host to feed and complete their life cycle and many factors can affect their success. Some hosts, such as bank voles (*Myodes glareolus*), develop acquired immunological resistance against *I. ricinus* ticks (Dizij & Kurtenbach, 1995) and have lower mean burdens compared to other small mammals of similar size (e.g. wood mice *Apodemus sylvaticus*) (Kiffner *et al.*, 2011b; Perez *et al.*, 2016; Tälleklint & Jaenson, 1997). Amongst mammals, males usually harbour more ticks compared to females (Brunner & Ostfeld, 2008; James, 2010; Ostfeld *et al.*, 1996; Ostfeld *et al.*, 2018a). This difference might be due to their bigger body mass (Harrison *et al.*, 2010; Hofmeester *et al.*, 2016; Kiffner *et al.*, 2011a), poorer immune functions (Klein, 2000; Tschirren *et al.*, 2003) and larger home ranges (Boyer *et al.*, 2010; Pollock *et al.*, 2012). It was found that some species harbour fewer ticks relative to their body size compared to other hosts (e.g. opossums, *Didelphis virginiana:* Keesing *et al.*, 2009; Levi *et al.*, 2016, mustelids: Hofmeester *et al.*, 2018) and this might be because they are able to groom them off more efficiently (Keesing *et al.*, 2009; Levi *et al.*, 2016; Shaw *et al.*, 2003). For instance, it was found that mustelids, which are competent reservoirs for *B. burgdorferi* s.l., have such low tick burdens that their participation in Lyme disease transmission cycle is thought to be negligible (Hofmeester *et al.*, 2018). Lastly, individual tick burden is also negatively

associated with host density (Deblinger *et al.*, 1993; Levi *et al.*, 2016; Ostfeld *et al.*, 1996).

In their review, Hofmeester et al. (2016), found that larval *Ixodes ricinus* ticks did not select specific hosts but fed on the most abundant host species present in an environment at low questing height, which is why thrushes and small mammals harboured the most larvae. Adult ticks, on the other hand, seemed to specifically select large size mammal, even if they are at a low density. Because adult female ticks are larger than immature life-stage, their host selection could be to avoid being groomed off by smaller sized vertebrates. While they found a positive correlation between host mass and nymphal burden, suggesting that nymphs could also be selecting larger size mammals, small mammals were the most important hosts for nymphal ticks due to their high abundance.

1.4.3. *B. burgdorferi* s.l. transmission

If they take a blood meal from an infected host, *I. ricinus* ticks can become infected with *B. burgdorferi* s.l. after 4-8 hours of feeding (Piesman *et al.*, 2001). The regulation and expression of different outer surface proteins by *B. burgdorferi* s.l. affect its survival and transmission as it enters the tick (Figure 1.6). *B. burgdorferi* s.l. spirochetes migrate to the tick midgut and the outer surface protein (Osp) A, expressed by *B. burgdorferi* s.l., binds to TROSPA receptors for OspA located in the tick midgut to ensure persistence of *B. burgdorferi* s.l. (de Taeye *et al.*, 2013; Hovius *et al.*, 2007; Pal *et al.*, 2004; Yang *et al.*, 2004). When an infected tick starts feeding on a host, OspA is downregulated while a second outer surface protein, OspC, is upregulated, to enable migration of *B. burgdorferi* s.l. from the midgut to the salivary gland (de Taeye *et al.*, 2013; Hovius *et al.*, 2007). The expression of OspC enables *B. burgdorferi* s.l. to bind to the tick salivary gland protein Salp15 and protects the pathogen from the host immune response (Ramamoorthi *et al.*, 2005; Schuijt *et al.*, 2008).

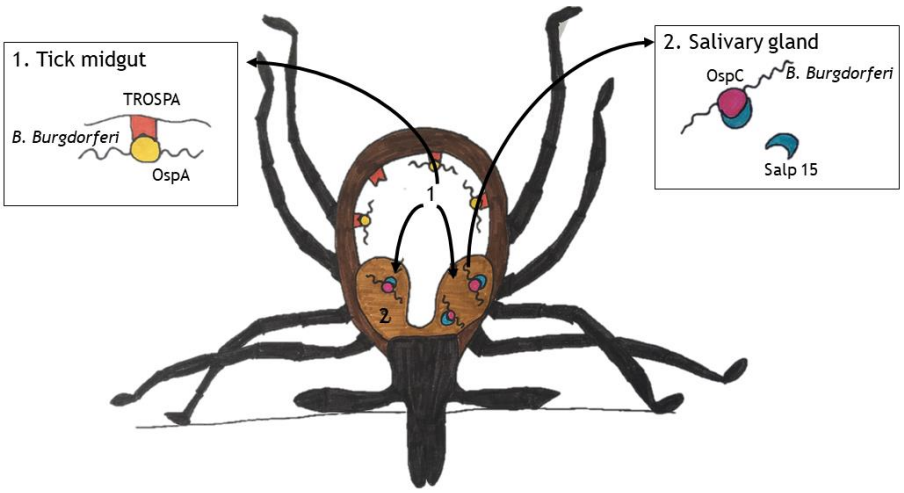


Figure 1.6: Schematic showing the tick-pathogen interaction. The outer surface protein A (OspA), expressed by *B. burgdorferi* s.l. binds to TROSPA receptors in the tick midgut (1). When the infected tick is feeding, OspA is downregulated and OspC is upregulated and *B. burgdorferi* s.l. migrates to the tick salivary gland where OspC binds to the tick salivary gland protein Salp 15 and protects the pathogen from the host immune response. Source: adapted from Hovius *et al.*, 2007.

Feeding on an infected host is not the only mechanism for naïve ticks to become infected. Indeed, transmission from an infected tick to an uninfected one can happen on an uninfected host, a mechanism known as co-feeding (Figure 1.7). In co-feeding, the host acts as a connection that brings ticks together spatially and temporally and allows pathogen transmission from an infected ticks to an uninfected one with a localised infection (Gern & Rais, 1996; Randolph, 2011; States *et al.*, 2017; Voordouw, 2015).

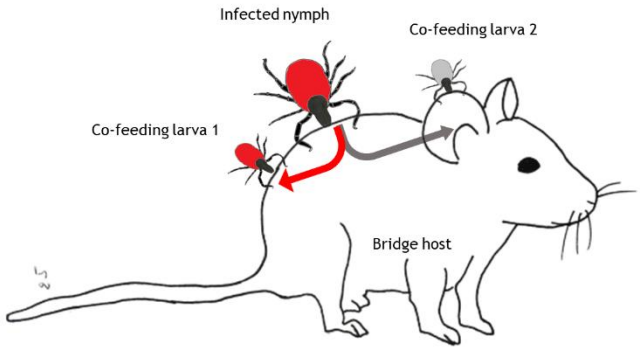


Figure 1.7: Conceptual figure illustrating co-feeding transmission on an uninfected host. Larva 1 acquires the pathogen from the infected nymph while larva 2 does not get infected because it is too far away from the infected nymphs. Source: Adapted from Voordouw, 2015.

1.4.4. Environmental factors and their effects on tick survival

Even though finding hosts and successfully feeding are necessary for ticks to complete their life cycle, tick survival also depends on many environmental factors affecting their questing behaviour and survival (e.g. temperature, humidity, ground vegetation).

Ticks are not active during winter and enter behavioural diapause until temperatures are high enough to ensure their survival (Randolph, 2004). Questing activity usually begins in early spring and, for a long time, it was thought that ticks emerged from their quiescence when temperatures reached 4-5°C (Clark, 1995; Duffy & Campbell, 1994). However, a study conducted by Gilbert *et al.* (2014) showed how ticks adapt to their environment and those from colder climate start questing at a lower temperature. For instance, 17.2% of ticks collected in Scotland were questing at 6°C while only 9.4% of those collected in France were questing at 7°C (Gilbert *et al.*, 2014). In Europe, nymphal questing activity reaches its peaks in late spring (April/May) and late summer (September/October (Hancock *et al.*, 2011; Hauser *et al.*, 2018; Perret *et al.*, 2004; Semtner & Hair, 1973) while larval activity has one peak in the summer in July/August (Dobson *et al.*, 2011a; Steele & Randolph, 1985).

While temperature is an important factor determining tick questing activity onset, the most limiting one for tick survival is humidity (Knap *et al.*, 2009; Milne, 1950; Randolph & Storey, 1999). Indeed, ticks are susceptible to desiccation and cannot survive for long periods of time in dry environments (Medlock *et al.*, 2013; Needham, 1991), which is probably why woodlands, providing constant leaf litter, shelter from wind and UV light are suitable environments and usually have much higher densities of ticks compared to open grasslands (Dobson *et al.*, 2011b; Estrada-peña, 2001; Lindström & Jaenson, 2003; Pfäffle *et al.*, 2013; Semtner & Hair, 1973; Vourc'h *et al.*, 2016). Forests in Europe also have more vertebrate species compared to other biomes (e.g. grasslands) (Danell, 1999; Friggens & Beier, 2010), which could further explain the difference in vector abundance observed.

1.4.5. Drivers of *Ixodes ricinus* expansion and Lyme disease emergence

The emergence of Lyme disease in recent decades in Europe and North America is likely to be a combination between changes in environmental factors, human behaviour and increased awareness. Land use changes and reforestation from the 1960's might have led to an increase in the number of deer, providing suitable hosts for ticks (Kurtenbach *et al.*, 2006; Medlock *et al.*, 2013; Spielman, 1994). The reduction in predators across much of Europe and North America might also have contributed to the increasing density of competent reservoir hosts and loss of biodiversity (Aguirre & Tabor, 2008; Levi *et al.*, 2012).

Environmental changes such as milder winters and earlier springs are also thought to have contributed to the increase in Ixodid tick abundance in parts of the world. For instance, *Ixodes ricinus* has expanded its geographical range northward in Sweden (Figure 1.8) and this spread has been correlated with milder winter temperatures and fewer days with snow cover (Jaenson *et al.*, 2012; Lindgren *et al.*, 2000; Lindgren & Jaenson, 2006).

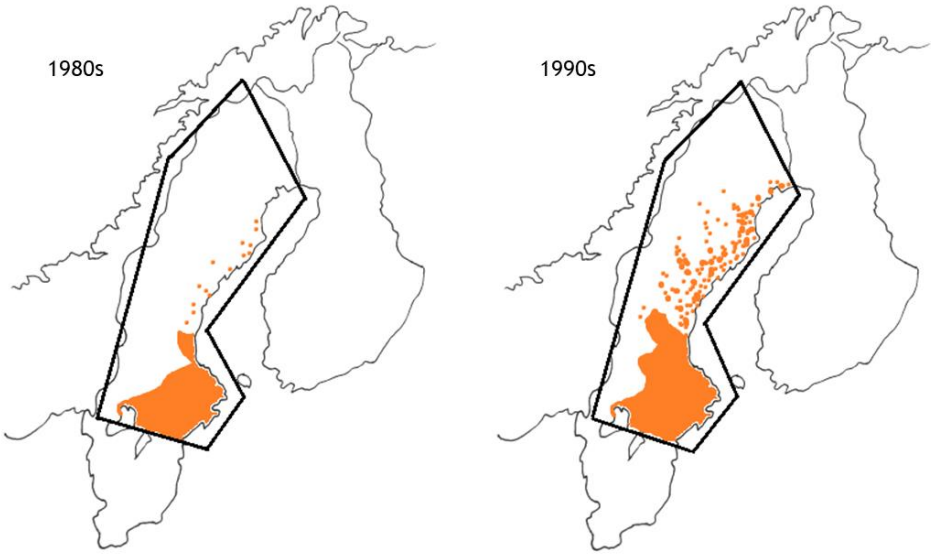


Figure 1.8: Map of Sweden representing locations where I. ricinus ticks were reported to be present (orange dots) in Sweden in the early 1980's (left) and 1990's (right). Source: Adapted from Lindgren *et al.*, 2000.

Changes in human behaviour and the rise of outdoor activities might have increased contact rates between humans and ticks (Hall *et al.*, 2017) and finally, increased awareness of Lyme disease by health practitioners and the general public are likely to have played a major role in the increased incidence observed (Mysterud *et al.*, 2016).

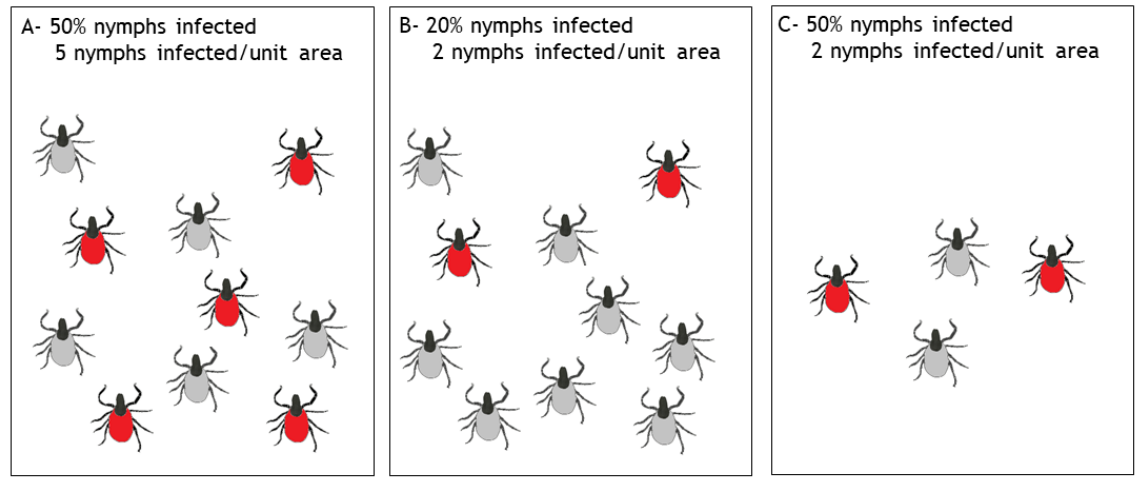
1.5. The vertebrate host community – effects on Lyme disease transmission risk

1.5.1. Lyme disease hazard – the density of infected nymphs

Lyme disease hazard is a combination between three components. The first one is disease hazard, which is defined as the density of questing *I. ricinus* nymphs infected with *B. burgdorferi* s.l. and is the combination between the abundance of ticks and *B. burgdorferi* s.l. infection prevalence (Allan et al., 2003; Kilpatrick et al., 2017; Logiudice et al., 2008) (Box 1). The second component, exposure, reflects the probability that an individual will be in contact with an infected tick and will be modulated by human behaviour. The third parameter is a person's vulnerability, which is the probability that an individual will develop an infection after being bitten by an infected tick[1].

In order to understand how disease hazard varies and identify environments with the highest risk, it is important to understand the factors affecting both the density of nymphs (DON) and nymphal infection prevalence (NIP). While some environmental factors (e.g. temperature, humidity, see Knap et al., 2009; Medlock et al., 2013; Milne, 1950; Randolph, 2004; Randolph & Storey, 1999) can affect tick survival and thus, DON, the presence and abundance of host species will have strong effects on both DON and NIP. The importance of a reservoir host in the Lyme disease cycle will depend on its abundance, its competency in transmitting *B. burgdorferi* s.l., its infection prevalence and the number of *I. ricinus* ticks it can feed (Kilpatrick et al., 2017).

Box 1: The importance of considering the density of infected nymphs



Lyme disease hazard is defined as the density of infected nymphs rather than the proportion of nymphs infected alone (Allan *et al.*, 2003; Kilpatrick *et al.*, 2017; Logiudice *et al.*, 2008). Scenarios A and C both have 50% of nymphs infected however, there are 5 nymphs infected per unit area in scenario A compared to 2 in scenario C, so the probability of being bitten by an infected tick is more than double in scenario A compared to C. In scenario B, 20% of nymphs are infected, which is more than half the prevalence in scenario C yet, both (B and C) have the same number of infected vector (2/unit area) and thus, similar risk for Lyme disease.

1.5.2. Tick reproduction hosts - how they drive tick abundance

Ungulate species, including deer, may affect *I. ricinus* abundance by providing blood meals for every tick life-stage (Gilbert *et al.*, 2012; Kugeler *et al.*, 2015; Li *et al.*, 2014; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014; Pichon *et al.*, 1999; Ruiz-Fons & Gilbert, 2010; Tälleklint & Jaenson, 1996; Vor *et al.*, 2010). For instance, roe deer feed, on average, 20.4 larvae compared to 18.5 nymphs and 25.3 adult female ticks (Hofmeester *et al.*, 2016; Tälleklint & Jaenson, 1997; Vázquez *et al.*, 2011; Vor *et al.*, 2010). While the contribution of deer species in feeding subadult tick life stages is likely to be dependent on host community, deer are referred to as tick reproduction hosts because they provide blood meals to adult females *I. ricinus*, which need to feed before laying their eggs (Gray, 1998; Jaenson *et al.*, 1994). Many studies have found that the abundance of questing nymphs is positively correlated with deer density (Gilbert *et al.*, 2012; Kugeler *et al.*, 2015; Li *et al.*, 2014; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014; Pichon *et al.*, 1999; Ruiz-Fons & Gilbert, 2010; Tälleklint & Jaenson, 1996; Vor *et al.*, 2010). To test

the importance of deer in supporting tick populations, some studies have experimentally excluded deer, either via fencing or culling and measured a large reduction in tick abundance (from a 50% to >90% reduction) (Gilbert *et al.*, 2012; Hofmeester *et al.*, 2017b; Kilpatrick *et al.*, 2014; Mysterud *et al.*, 2016).

1.5.3. Host competency - role in driving *B. burgdorferi* s.l. prevalence

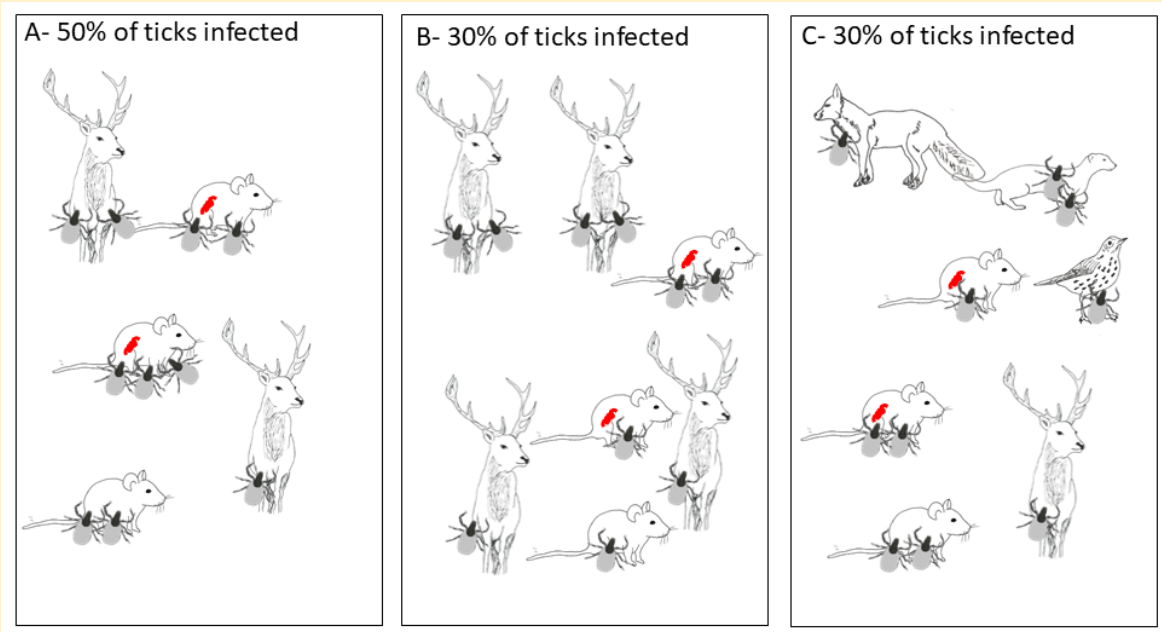
Nymphal infection prevalence is the second parameter determining the density of infected nymphs in the environment and depends on the number of larvae successfully feeding and moulting into nymphs and the proportion of these which are infected with *B. burgdorferi* s.l.

The number of larvae successfully feeding depends on the abundance of hosts (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2006; Perez *et al.*, 2016; Rosà *et al.*, 2007) and which host species they fed from, as some hosts have acquired resistance to ticks (Dizij & Kurtenbach, 1995) or are more able to groom ticks off (Keesing *et al.*, 2009; Levi *et al.*, 2016; Shaw *et al.*, 2003), as well as other factors influencing tick-host interactions (e.g. vegetation height for tick questing). Regarding NIP, some studies have found that the proportion of nymphs infected with *B. burgdorferi* s.l. correlates with competent reservoir abundance the previous year, when they fed as larvae (Kilpatrick *et al.*, 2017; Mysterud *et al.*, 2016; Vuong *et al.*, 2017). More specifically, and due to associations between some genospecies and hosts species, NIP for each genospecies is correlated with the density of their associated hosts the previous year (James, 2010; Krawczyk *et al.*, 2020; Takumi *et al.*, 2019). However, the infection prevalence rate in the reservoir host population as well as other factors that might influence reservoir host abundance will influence nymphal infection prevalence and this will be investigated in Chapter 4.

While other hosts, such as cattle and deer can feed large number of ticks, they are non-competent hosts and therefore, ticks feeding on them will not get infected (Kurtenbach *et al.*, 2002; Pacilly *et al.*, 2014; Richter & Matuschka, 2010; Rosef *et al.*, 2009). Although transmission of *B. burgdorferi* s.l. through tick co-feeding on non-competent sheep has been demonstrated before (Ogden *et al.*, 1997), it is difficult to quantify on wild ungulates but could occur.

Therefore, if the host community is mainly composed of non-competent hosts and because *I. ricinus* ticks are generalists, the probability of them feeding on these hosts rather than on competent reservoir hosts will increase and thus, infection rate in the tick population should drop, a concept called the dilution effect (see Box 2). Since the theoretical basis for the dilution effect was outlined (Norman *et al.*, 1999), several studies have tested this theoretically (Cagnacci *et al.*, 2012; Logiudice *et al.*, 2008; Ostfeld & Logiudice, 2003; Rosà *et al.*, 2003) and empirically (Bouchard *et al.*, 2013; Cagnacci *et al.*, 2012; Huang *et al.*, 2018; Kilpatrick *et al.*, 2014; Krasnov *et al.*, 2007; Levi *et al.*, 2016; Mysterud *et al.*, 2016; Pichon *et al.*, 1999; Ruyts *et al.*, 2018; Takumi *et al.*, 2019). While some studies have found significant relationships between high deer density and a decrease in pathogen prevalence in questing nymphs (Rosef *et al.*, 2009; Ruiz-Fons *et al.*, 2012; Vourc'h *et al.*, 2016), the role of deer as a dilution host is still contradictory as other studies did not observe any association (Pichon *et al.*, 1999). This may be due to these studies sampling from a limited range of deer density and only sampling deer, without quantifying the abundance of other host species present. Thus, rather than only considering the density of the non-competent host (deer), the ratio between non-competent (deer) and competent reservoir hosts (small mammals, birds) in an environment may be the more relevant parameters (Rosà *et al.*, 2003). This has not been tested before and thus, I have designed a study that will address this (Chapter 2).

Box 2: The dilution effect theory



The dilution effect theory was proposed in the early 2000's when researchers hypothesised that an increase in low competency host density in an ecosystem could reduce disease transmission by diverting the pathogen from competent reservoir hosts (LoGiudice *et al.*, 2003; Norman *et al.*, 1999; Ostfeld & Keesing, 2000a, 2000b; Schmidt & Ostfeld, 2001). To illustrate this theory using Lyme disease, let us take an ecosystem that has a high density of competent small mammals (Block A). In this case, ticks will be shared amongst the hosts available and a large proportion will feed onto infected small mammals, resulting in 50% of ticks being infected. If the density of non-competent deer increase (Block B), the probability of ticks feeding on them will rise, resulting in a lower infection rate (30%) (Rosef *et al.*, 2009; Ruiz-Fons *et al.*, 2012; Vourc'h *et al.*, 2016). Some studies have suggested that an increase in biodiversity (Block C) could result in a rise in the proportion of non-competent hosts, resulting in a lower infection rate (only 30% in this case). While this has been shown in North America (LoGiudice *et al.*, 2003; Ostfeld & Keesing, 2000a; Werden *et al.*, 2014), this effect is still controversial (Randolph & Dobson, 2012) and will not be tested in this thesis.

While conducting field studies, it is only possible to get partial measures of different reservoir host species contribution to Lyme disease system. Indeed, tick survival from feeding onto different hosts is not accounted for and the proportion of ticks feeding on different hosts is not quantified but related to host abundance in an ecosystem. The blood meal analysis methods will allow researchers to better understand the contribution of each species but there is a need to improve their sensitivity before that is possible.

1.5.4. The host community - interactions between species and the environment

Host community composition plays a crucial role in shaping Lyme disease hazard in an environment via vector-host-pathogen interactions. Therefore, it is also important to understand the drivers of host community composition and the interactions between host species and their environment as it could affect species' abundance.

Pulsed availability of resources, which can occur regularly in nature, can strongly affect consumers' populations (Clotfelter *et al.*, 2007; Jones *et al.*, 1998; McShea, 2000; Ostfeld & Keesing, 2000c; Touzot *et al.*, 2020). Small mammals, with their short generation time and fast turn-over, rapidly respond to variations in resources, which usually results in higher densities of individuals in good body condition (Nie & Liu, 2005; Rémy *et al.*, 2013; Taitt & Krebs, 1981). The repercussions that this could have on tick burden and nymphal infection prevalence have been studied in the US (Ostfeld *et al.*, 2001, 2006) and the Netherlands (Krawczyk *et al.*, 2020; van Duijvendijk, 2016) but no study has been conducted in the UK and I will investigate the effect of a change in resources on small mammal populations and Lyme disease hazard in Chapter 4.

Interspecific competition by interference or exploitation can also play a role in shaping host communities and has been shown to affect populations in several deer species (Focardi *et al.*, 2006; Latham *et al.*, 1996; Latham & Staines, 1997). This can be important for Lyme disease as several species can harbour different tick burdens (Hofmeester *et al.*, 2016) and I will investigate a potential interspecific competition between roe and red deer and its repercussion on Lyme disease hazard in Chapter 2.

Roe and red deer can also have major impacts on their environment and host community through browsing pressure. Indeed, grazing by deer can result in shorter and sparser vegetation (Buesching *et al.*, 2011), which could have an impact on tick questing behaviour (Mejlon & Jaenson, 1997) but, more importantly, on small mammals. Indeed, loss of cover from predators and trampling by ungulates can lead to fewer small mammals (Dirzo *et al.*, 2014;

Flowerdew & Ellwood, 2001; Keesing & Young, 2014; Smit *et al.*, 2011; van Wieren & Bakker, 2008) but the repercussion that this could have on Lyme disease hazard has not been studied and will be tested in Chapter 3.

Other forms of anthropogenic pressure such as the creation of hiking trails, hunting pressure and more generally human activity can impact species distribution and their use of habitat (Bonnot *et al.*, 2013; Coppes *et al.*, 2017; Hewison *et al.*, 2001; Scholten *et al.*, 2018). However, the impacts that it could have on tick density, small mammal communities and Lyme disease hazard remain unclear and I will investigate these effects in Chapter 2.

1.5.5. Concluding remarks on the effect of the vertebrate host community on Lyme disease transmission

The Lyme disease epidemiological system is very complex, with several interacting elements. Therefore, I tried to summarize how Lyme disease hazard might be affected by host and environmental factors in the figure below (Figure 1.9).

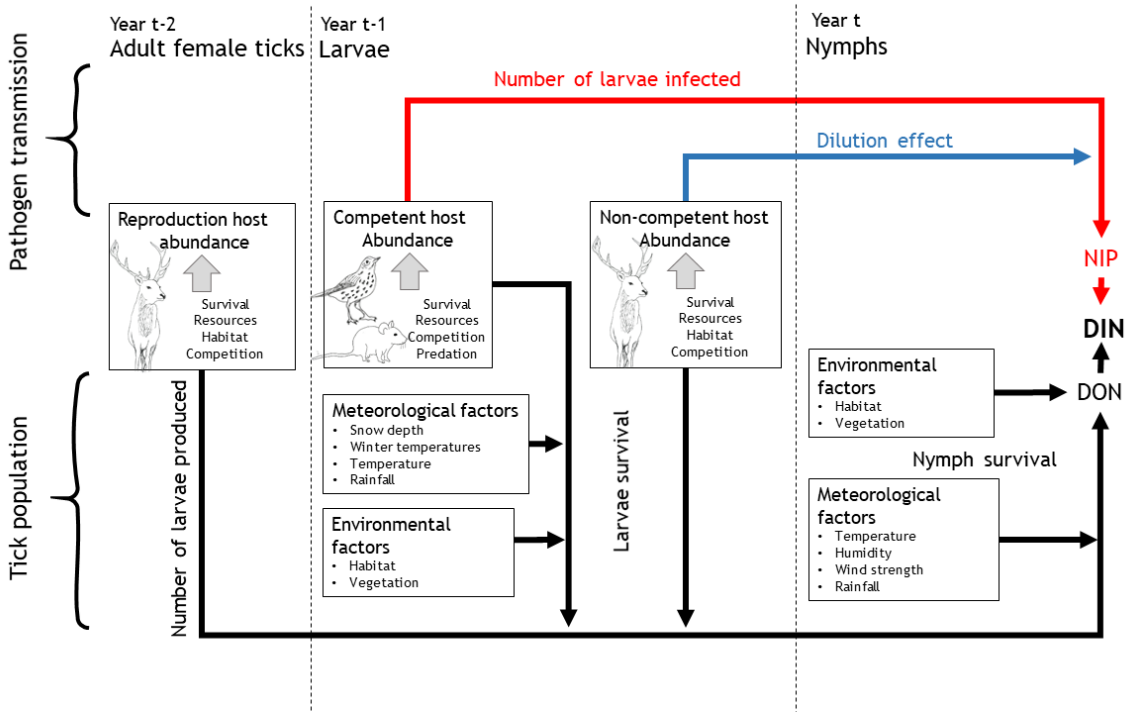


Figure 1.9: Conceptual figure illustrating the various factors that could affect the density of infected nymphs (DIN) at $year_t$ based on environmental factors and host community composition at $year_{t-1}$ and $year_{t-2}$. Black arrows represent the effects of these factors at $year_t$ $year_{t-1}$ and $year_{t-2}$ on the density of nymphs (DON), the red arrow represents the positive effects that host community composition at $year_{t-1}$ (when larvae fed) can have on nymphal infection prevalence (NIP) and the blue arrow the negative effects that hosts at $year_{t-1}$ can have on NIP.

Host community composition plays a major role in determining Lyme disease hazard. Competent reservoir hosts such as birds and small mammals are associated with NIP, depending on infection prevalence rates in reservoir hosts, and the number of larvae that will successfully feed and moult into nymphs. Deer could play three different parts in the epidemiological cycle; first, a high density of deer could lower NIP by diverting ticks from feeding on competent reservoir hosts (dilution effect). Secondly, they are likely to drive tick populations, thus increasing the density of ticks. Thirdly, they could reduce Lyme disease hazard by reducing the abundance of competent reservoir hosts through their impacts on the vegetation but this is not typically measured in empirical studies.

1.6. Thesis aims and general outline

In this thesis, I used several approaches to investigate how host community composition affects *I. ricinus* abundance, infection prevalence with *B. burgdorferi* s.l. and thus, Lyme disease hazard in Scottish woodlands. I also looked at the interactions between different host species and between hosts and their environments. This was approached by designing a large cross-sectional field study to investigate how Lyme disease hazard varied along the full gradient of deer densities while simultaneously measuring small mammal abundance (Chapter 2). I also used experimental studies to test the cascading effects of high deer density on Lyme disease hazard, via their effects on vegetation and small mammals (Chapter 3) and testes the effects of food supplementation on wood mouse abundance and Lyme disease hazard (Chapter 4).

Chapter 2: The objective of this study was to test the impact of the interaction between deer and small mammals on Lyme disease hazard. To do so, I conducted a cross-sectional survey at 28 woodland sites specifically selected to cover the full range of deer densities, surveyed ticks and estimated the abundance of small mammals. After assessing the effect of deer and small mammals on DIN, I aimed to identify the different mechanisms involved separately; I first investigated how the density of nymphs (DON) varied depending on the abundance of deer (tick reproduction hosts) in the environment. Then, I assessed how the prevalence of *B. burgdorferi* s.l. varied depending on the ratio of competent reservoir hosts (small mammals) to non-competent hosts (deer). Lastly, I used the data to

examine how different host species interact (i.e. spatial distribution of roe and red deer and whether high deer densities negatively impact small mammals) and whether anthropogenic pressures affect hosts, which has implications for potential cascading effects on *I. ricinus* abundance and *B. burgdorferi* s.l. prevalence.

Chapter 3: The objective was to test the cascading effects of high deer density on Lyme disease hazard through effects on vegetation and small mammal communities. I used an experimental design consisting of replicated fenced exclosure plots in a landscape-scale moorland area of a deer farm (with very high deer density), thus creating a contrast between the absence of deer and high deer density. First, I tested the role of deer as tick reproduction hosts and I assessed the effects of high deer density on tick density compared to deer exclusion plots. Secondly, I investigated the role of high deer density in lowering *B. burgdorferi* s.l. prevalence through a dilution effect and through the effects of deer on vegetation and small mammal communities.

Chapter 4: To assess the impacts of a change in competent reservoir host (wood mice) abundance through food supplementation on Lyme disease hazard, I used an experimental design in which trapping grids were either supplemented with food or left as controls. Wood mouse abundance was estimated, along with tick burdens on wood mice, questing tick density and *B. burgdorferi* s.l. prevalence. I then investigated the effects of a change in food resources on the abundance of wood mice, tick infestations, the abundance of *I. ricinus* nymphs, *B. burgdorferi* s.l. prevalence, and DIN.

Chapter 5: This chapter discusses the results and limitations from previous chapters and their implications. I also discuss limitations in the studies conducted, gaps in knowledge and I make suggestion for next steps for research in the field that came to light during this PhD.

2. The impacts of host community composition on Lyme disease hazard – a cross-sectional survey in Northeast Scotland

2.1. Abstract

To better comprehend the factors leading to emergence of zoonoses, it is important to understand the interplay between environmental factors, wild reservoir hosts and pathogens and host community composition plays an important role in determining the spread of these zoonoses. When considering vector-borne pathogens, disease hazard is defined as the density of infected vectors. In the case of Lyme disease, a bacterial zoonosis caused by *Borrelia burgdorferi* s.l. and transmitted by Ixodid ticks, disease hazard will depend on factors affecting tick densities and prevalence of *B. burgdorferi* s.l. Small mammals are competent reservoir hosts for one of the *B. burgdorferi* s.l. genospecies, *B. afzelii*, driving tick nymphal infection prevalence (NIP), while deer are non-competent hosts but are the most important tick reproduction hosts, driving the density of nymphal ticks (DON). Therefore, high density of deer could increase the density of infected nymphs (DIN) by increasing DON or reduce DIN through a dilution effect, as a larger proportion of ticks should feed on non-competent deer. This study aimed to investigate how the ratio of small mammals and deer affected DIN. I conducted a cross-sectional survey across 28 sites in Northeast Scotland between 2017 and 2019 and data on host abundance (deer, small mammals), *Ixodes ricinus* tick density and *B. burgdorferi* s.l. prevalence were collected at each site. DON was positively correlated with deer density and I observed a dilution effect by roe deer on *B. afzelii*, regardless of small mammal abundance. Regarding disease hazard, DIN for *B. afzelii* was positively associated with both roe and red deer density. There was a negative relationship between roe and red deer density and roe deer density was also negatively impacted by human disturbance, which could affect DIN. Regarding small mammals, their abundance was lower when deer density was high, suggesting a cascading effect of high deer density that is likely to affect DIN. Results showed that the role of deer in driving tick populations up can outweigh their role in diluting NIP and DIN for *B. afzelii* was driven by deer density.

2.2. Introduction

The emergence of infectious diseases depends on many factors ranging from environmental changes expanding reservoir hosts distribution ranges (Githeko *et al.*, 2000) to increased contact rates between humans and wild reservoir hosts due to habitat fragmentation, urbanization and meat consumption (Alexander *et al.*, 2015; Engering *et al.*, 2013; Parrish *et al.*, 2008; Schrag, Stepanie & Wiener, 1995). Zoonoses represent 75% of emerging infectious diseases (Rhyan & Spraker, 2010) and spread to humans after spill over events (Daszak *et al.*, 2000; Engering *et al.*, 2013). Because many of these pathogens are maintained in wildlife reservoir hosts, host community composition plays an important role in determining the risk of multi-host zoonotic pathogens for humans.

In the case of vector-borne diseases, for which living organisms transmit pathogens between various hosts, disease hazard is defined as a combination of vector abundance and pathogen prevalence and will increase with the density of infected vectors (Allan *et al.*, 2003; Ezenwa *et al.*, 2006; Kilpatrick *et al.*, 2017). Therefore, vector survival and thus, abundance plays an important role in determining disease hazard and is determined by a wide range of factors including climatic variables (e.g. temperature, humidity) (Bertrand & Wilson, 1996; Tun-Lin *et al.*, 2000), and environmental landscape features (e.g. ground vegetation, elevation, habitat type) (Gilbert, 2010; Sweeney *et al.*, 2006; Walker *et al.*, 2001). When vectors are blood feeding arthropods, the abundance of suitable hosts in the environment can determine how many vectors successfully complete their life cycle and many studies have shown that host abundance can drive vector populations (Gilbert *et al.*, 2012; Krasnov *et al.*, 2002; Minakawa *et al.*, 2002; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014).

Pathogen prevalence, on the other hand, is positively correlated with both the abundance of competent reservoir hosts, which maintain and transmit pathogens, and the number of vectors feeding on these competent transmission hosts (Ostfeld *et al.*, 2006; Telfer *et al.*, 2005; van Duijvendijk, 2016; Vuong *et al.*, 2017). In contrast, vectors feeding on non-competent hosts will not acquire the pathogen and it has been hypothesised that an increase in non-competent hosts could, theoretically, reduce pathogen prevalence by diverting vectors away from feeding

on competent reservoir hosts, a concept called the dilution effect (Keesing *et al.*, 2010; LoGiudice *et al.*, 2003; Norman *et al.*, 1999; Ostfeld & Keesing, 2000a, 2000b; Schmidt & Ostfeld, 2001). Disease hazard will thus depend on the abundance of vector reproduction hosts and the combination of both competent reservoir hosts and non-competent hosts.

Lyme disease, the most prevalent vector-borne disease in humans in the Northern hemisphere (Steere *et al.*, 2004; Walter *et al.*, 2017), is a multi-host vector-borne zoonosis caused by a range of bacteria belonging to the genus *Borrelia burgdorferi* sensu lato (hereafter referred to as *B. burgdorferi* s.l.). It is an ideal system to investigate the effects of host community composition on disease hazard because the vector feeds on a wide range of competent reservoirs and non-competent hosts. In Europe, Lyme disease is primarily transmitted by *Ixodes ricinus* ticks (Piesman & Gern, 2004). *I. ricinus* ticks undergo three stages of life (larva, nymph and adult) and each stage must take a blood meal in order to moult into the next life-stage (Oliver, 1989). Larvae and nymphs feed on most terrestrial vertebrate species while adult females usually take a blood meal from large size mammals before laying their eggs. Deer are an important source of blood meals for both immature ticks and adult females and are known to drive tick abundance (Gilbert *et al.*, 2012; Ruiz-Fons & Gilbert, 2010; Tälleklint & Jaenson, 1996). Although deer are well documented to be the primary drivers of *I. ricinus* populations, small mammals can also play a role by feeding large numbers of larval ticks, and two studies have shown a positive correlation between nymph density and the density of small mammals the previous year (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2006).

Small mammals (bank voles *Myodes glareolus*, wood mice *Apodemus sylvaticus* and shrews *Sorex* spp.), birds and squirrels (*Sciurus* spp.) are common competent reservoir hosts for the various genospecies of *B. burgdorferi* s.l. in Northern Europe while roe (*Capreolus capreolus)* and red deer (*Cervus elaphus*) are non-competent hosts for *B. burgdorferi* s.l. transmission. Four different genospecies of *B. burgdorferi* s.l. are present in the UK, each having different associations with reservoir hosts. *B. afzelii* is maintained by small mammals (Hanincová *et al.*, 2003a; Kybicová, Kurzová and Hulínská, 2008) while *B. valaisiana* and *B. garinii* are primarily associated with avian species (Hanincová *et al.*, 2003b; Heylen *et al.*, 2014). *B. burgdorferi* sensu stricto, the fourth genospecies, is a generalist and

can be maintained by any competent host (Heylen *et al.*, 2014; Kurtenbach, Sewell, *et al.*, 1998; Norte *et al.*, 2012; Richter *et al.*, 2000). There is evidence for the role of deer as dilution hosts and several studies have shown that *B. burgdorferi* s.l. prevalence can decrease with increasing deer density (Huang *et al.*, 2018; Rosef *et al.*, 2009; Vourc'h *et al.*, 2016). This could result in lower disease hazard when the role of deer in diverting ticks from feeding on competent reservoir hosts overcomes their role in amplifying tick density (Huang *et al.*, 2018). In Europe, it is also important to consider roe and red deer as two different hosts as their differences in ecology, social behaviour and habitat usage might affect tick density and pathogen prevalence in different ways (Borkowski & Ukalska, 2008; Latham *et al.*, 1996; Welch *et al.*, 1990).

The density of infected nymphs (DIN), representing Lyme disease hazard, will therefore be affected not only by environmental and climatic factors but also by the abundance of competent reservoir hosts and non-competent hosts. In this context, it is important to not consider each host species individually, but to investigate their combined role as the ratio of competent reservoir hosts (small mammals, birds) and non-competent hosts (deer). Although some empirical studies have shown a positive association between DIN and competent reservoir host abundance (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2018b; Takumi *et al.*, 2019), fewer have investigated the effects of deer density on DIN (Takumi *et al.*, 2019; Vourc'h *et al.*, 2016). While two theoretical studies explored the effect of the ratio of competent and non-competent hosts on disease hazard (Bolzoni *et al.*, 2012; Rosà *et al.*, 2003), no empirical study has confirmed those predictions and thus, I will test this in this study.

Species interactions must also be considered when studying the effect of host communities on disease hazard. Indeed, interspecific competition plays a major role in shaping host communities and has been shown to alter foraging behaviour and change relative abundance of several deer species (Ferretti *et al.*, 2011; Focardi *et al.*, 2006; Richard *et al.*, 2010). Similarly, anthropogenic pressure can lead to spatial variations in the perception of risk by prey, resulting in a landscape of fear (Laundré *et al.*, 2010) and this could have repercussions on multiple trophic levels, including disease hazard. However, these effects have not been studied for Lyme disease. Although browsing pressure by ungulates is known to affect

vegetation structure (Buesching *et al.*, 2011; Pellerin *et al.*, 2010) and impact small mammal and bird communities (Allombert *et al.*, 2005; DeCalesta, 1994; Flowerdew & Ellwood, 2001; McShea & Rappole, 2000; Smit *et al.*, 2011; van Wieren & Bakker, 2008), the potential repercussions on pathogen prevalence and disease hazard are unknown.

In this study, I aimed to empirically assess how host community composition affects Lyme disease hazard. To do so, I conducted a cross-sectional survey across 28 sites in Northeast Scotland, selected to specifically cover the full range of deer densities, while also covering a range of small mammal abundance. This study focussed on bank voles and wood mice as *B. afzelii*, the genospecies associated with small mammals in the most prevalent in Scotland (James *et al.*, 2013, 2014; Millins *et al.*, 2016). Specifically, my goal was to test the impact of the interaction between deer and small mammals on DIN and to identify which densities led to the highest DIN. I predicted that DIN should be highest when both deer and small mammals are present at high density and lowest when both occur at low densities (Figure 2.1). I could not make predictions regarding Lyme disease hazard for the entire range of deer and small mammal densities due to the opposing effects of deer on DIN as they drive tick populations up but might lower pathogen prevalence through a dilution effect and by affecting small mammal communities.

Because Lyme disease hazard depends on the density of nymphs (DON) and *B. burgdorferi* s.l. nymphal infection prevalence (NIP), I investigated how host abundance affected these two components. I predicted that DON should be positively correlated with the density of both roe and red deer (Figure 2.1). I also examined larval burden on small mammals (Hanincová *et al.*, 2003a; Perez *et al.*, 2016; Tälleklint & Jaenson, 1994), as nymph abundance could be linked to the number of larval ticks successfully feeding the previous year.

Regarding NIP for *B. burgdorferi* s.l., I hypothesised that NIP should decrease as a function of increasing roe and red deer densities due to their role as dilution hosts. I also predicted that NIP for *B. afzelii* should be higher in sites presenting high abundance of small mammals. However, I still expected to observe a reduction in prevalence when deer densities increased (Figure 2.1).

Lastly, I looked at how hosts were affected by various factors as this might affect their abundance and thus, their impacts on NIP and DON. I predicted that small mammals should be affected by environmental features (e.g. ground vegetation, type of habitat) and deer density. Regarding roe and red deer, I expected to see a landscape of fear created by hunting pressure and human disturbance but it might not affect each species the same way (Bonnot *et al.*, 2013; Coppes *et al.*, 2017; Hewison *et al.*, 2001; Scholten *et al.*, 2018). Finally, I presumed that I should observe interspecific interactions between roe and red deer (Ferretti *et al.*, 2011; Focardi *et al.*, 2006; Richard *et al.*, 2010) and both species should only be found co-existing at high densities if resources are sufficient.

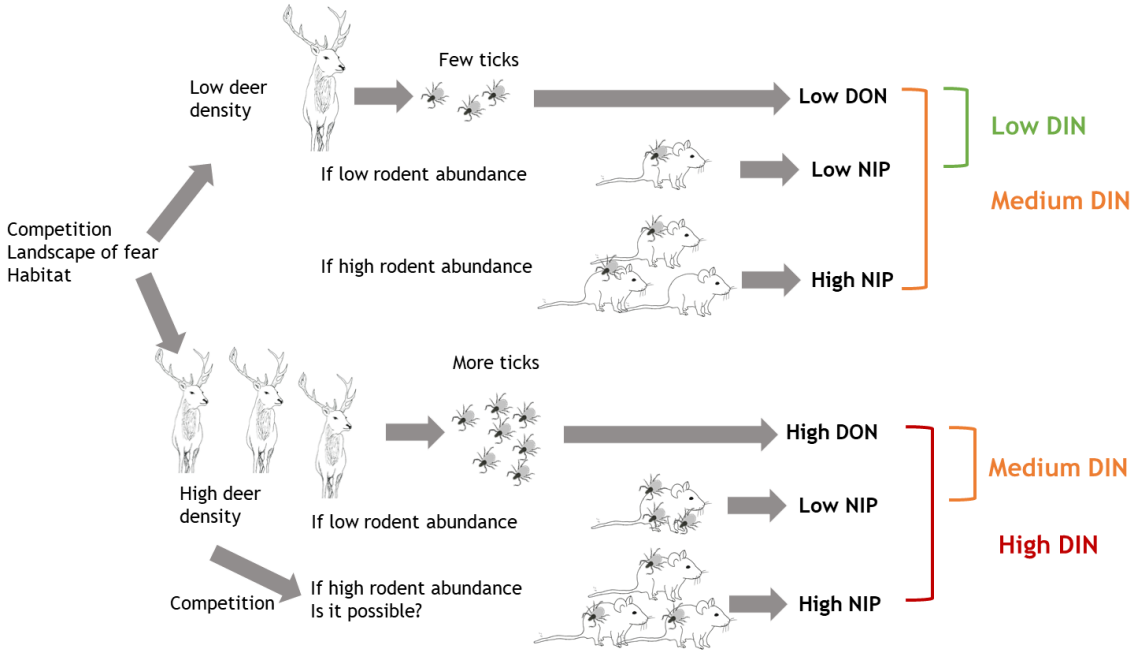


Figure 2.1: Conceptual figure illustrating the predicted effects of deer and small mammal densities on the density of nymphs (DON), nymphal infection prevalence (NIP) and Lyme disease hazard (DIN). Due to the complexity of the interactions, the system was simplified in separating deer and small mammals in low or high density. However, I am expecting more complexity with varying densities of deer and small mammals.

2.3. Materials and methods

2.3.1. Study sites

To assess the effects of host community composition on Lyme disease hazard, I selected 28 sites located in Aberdeenshire, North East Scotland (central point: 57.000°N, 2.700°W) (Figure 2.2). Sites were selected specifically to have a wide range of deer densities, including zero deer by using two woodlands that were fenced (sites GLS and GSB), based on a pilot study conducted in September 2016. As this study focused on the epidemiological cycle of Lyme disease in wild reservoir hosts, I chose sites in which sheep and cattle were absent. To minimise the effects of environmental variability on Lyme disease hazard, all coniferous (n=21), deciduous (n=3) and mixed (n=4) woodlands were selected within the same altitudinal range (112-274m), as tick density varies with altitude (Gilbert, 2010; Medlock *et al.*, 2013), no more than 50 km apart but separated from one another by at least 1km to ensure their independency. Each site was surveyed for two consecutive years (19 sites were surveyed in 2017 & 2018 and 9 in 2018 & 2019). During the first year of each site's survey, deer density and small mammal abundance were estimated and questing ticks were collected. The following year, deer density was estimated a second time and ticks were collected to estimate the prevalence of *B. burgdorferi* s.l. one year after small mammal trapping took place. I used this one-year lag between small mammal trapping and *B. burgdorferi* s.l. prevalence estimates because small mammal populations can fluctuate (Flowerdew & Gardner, 1978; O'Mahony *et al.*, 1999) and the questing nymphs collected in year 2 potentially fed on small mammals and acquired *B. burgdorferi* s.l. infection from small mammals in year 1. Forestry work started at two sites (KNW and BOD) halfway through the study and therefore, they were excluded for prevalence estimates as ticks could not be collected the second year.

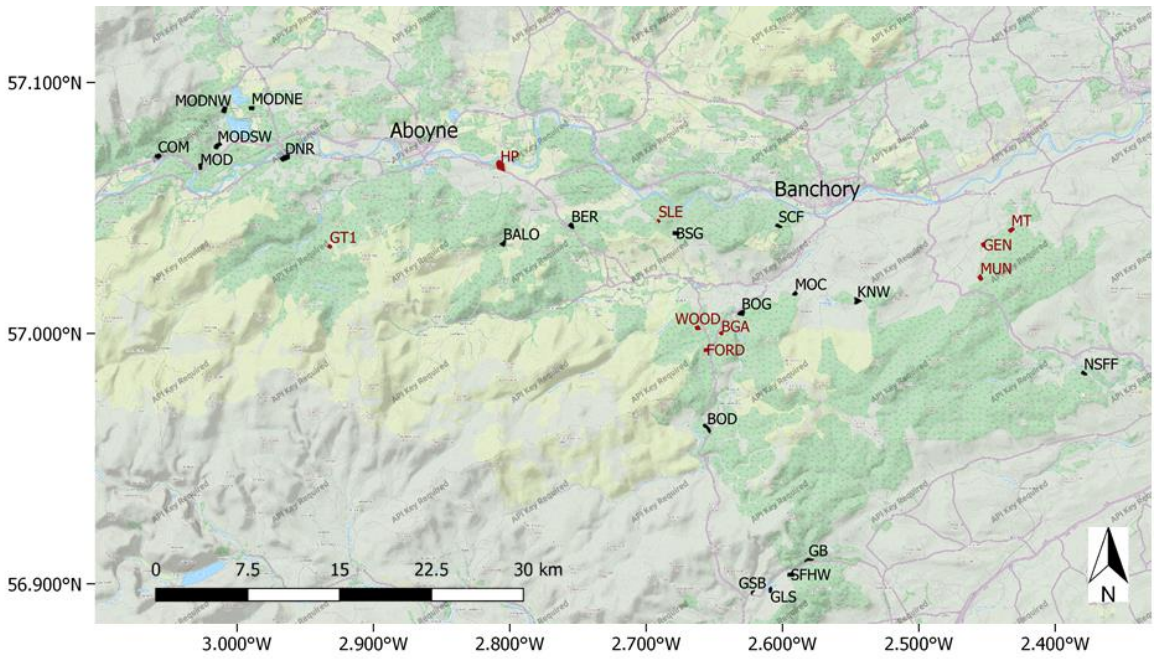


Figure 2.2: Map of part of Aberdeenshire, NE Scotland, representing the woodland sites used for this study. Sites in black were surveyed in 2017 & 2018 and sites in red in 2018 & 2019.

2.3.2. Estimation of deer density

An index for deer density was estimated in May for two consecutive years using the standing crop plot count method (Acevedo *et al.*, 2010; Mayle *et al.*, 1999). Pellet groups were counted in May because the ground vegetation was short and pellets could be easily detected. Within each site, twenty 10m long and 1m wide linear transects, separated from one another by at least 20m, were randomly generated using QGis software (QGIS Development Team, 2016). The start of each transect was identified using GPS coordinates and the bearing was determined by alternately selecting a north, south, east and west heading. The number of red and roe deer pellet groups were counted along each transect (for $10m^2$) and date, habitat and ground vegetation were also recorded. Habitat (coniferous, deciduous or mixed woodland) was recorded at the site level while ground vegetation was classified into four categories: (1) grasses and herbaceous species (Grass), (2) *Ericaceous* and *Vaccinium* species (EV), (3) moss species (Moss) and (4) bracken and ferns (BF) (Millins, 2016) and the dominant category for each transect was recorded.

An index for red and roe deer density for each site was calculated by adapting PELLET® (Mandujano, 2014), a semi-automatic procedure in Microsoft Excel®, in R using the following formula:

$$D_{ind} = \frac{D_{pg}\ x\ N_p}{Def\ x\ Decay}$$

Where D_{ind} is the density of red or roe deer per km^{-2}, D_{pg} the average number of pellet groups per site, N_p is a constant to convert the sampled surface into square kilometres, *Def* the defecation rate and *Decay* is the number of days for pellets to decay. This calculation incorporated variations in decay and defecation rates, taken from the literature to allow for a more accurate estimation of population density (defecation rates for red deer: 20.46, 24.88 and 29.3 per day, Mitchell *et al.*, 1983; Mitchell & McCowan, 1984, for roe deer: 17.08, 19.33 and 21.58 per day Mitchell *et al.*, 1985). Minimum decay was 189 days and maximum decay was 416 days (Mayle *et al.*, 1999).

To confirm whether this method was accurate enough to be used in this study, I tested it by conducting pellet group counts in a deer farm (30.81 red deer/km^{-2}) and comparing the resulting density estimate with the known density of deer (32.47 red deer/km^{-2}) in the enclosure.

I assessed the stability of deer density at each site since lag effects of deer density could affect tick abundance. Indeed, deer feed all life stages of *Ixodes ricinus* and could be linked to questing nymph density by feeding adult female ticks two years prior to sampling questing nymphs, and by feeding larval ticks one year prior to sampling nymphs. I performed a two-sample permutation test with Bonferroni correction on pellet count data to assess how stable deer density was between both years of collection. Results showed that deer density remained stable at each site as all p-values were above 0.5 (Appendix A, S3).

2.3.3. Estimation of small mammal abundance

I used two methods to estimate the abundance of small mammals in the study area. First, small mammal abundance (number of bank voles and wood mice captured per 100 trap nights) was quantified at each site by using a live-trapping method (Bouchard *et al.*, 2013; Quéré *et al.*, 2000) (19 sites surveyed in 2017 and 9 in 2018) under the Home Office Regulations Project license 70/8543 and personal license I2B15B1E9. To minimize the effect of seasonal variations in small mammal populations, trapping was done at all study sites within 40 days in June and July. Ten to fifteen 100m long transects separated by 10m were set up at each site. Ten non-selective Sherman traps (16 x 5 x 6.5 cm HB Sherman Inc., Tallahassee, Flor.) were installed every ten meters on each trapping line and baited with oats for two nights. Traps were equipped with shrew holes, allowing shrews (*Sorex* spp.) to escape as it was not logistically possible to meet the license requirements for shrew trapping. Traps were left inactivated during the day and were activated after 1700 hours every evening. Bedding material was provided for comfort and to reduce the risk of hypothermia. Traps were checked every morning before 1000 hours and species (bank vole or wood mouse, the only two species found in Scottish woodlands), sex, weight and age category (juvenile or adult) of each individual were recorded. In addition, ticks were counted around the head and ears, and engorged ticks were collected and stored in 100% ethanol. A skin biopsy was taken from each individual upon first capture using an ear punch and stored in 100% ethanol (Sinsky & Piesman, 1989). All trapped individuals were released at the capture site.

The second method I used to assess small mammal abundance consisted in recording vole signs (tunnels, holes and latrines) at each site in May on the transects used for the deer density estimation. The average number of vole signs observed per $10m^{-2}$ was reported.

Because each method was biased by ground vegetation type (small mammal signs were easier to detect in grass whereas live trapping success was much lower in grass than in other ground vegetation types; see results), it was necessary to create an index of small mammal abundance which combined the vole sign observation index and small mammal trapping data. First, I divided each site in

either low or high small mammal abundance for each index (i.e. small mammal trapping and vole sign) based on their distribution, as a clear gap between low and high density could be seen, before combining them. If a site scored a high category for at least one index, it was defined as a high small mammal abundance site whereas sites which scored low categories for both indexes were identified as low small mammal abundance (Appendix A, S4). For instance, if a site scored low from live trapping but high from vole sign, it was classified as high small mammal abundance. Due to the small number of small mammals captured, I decided to group bank voles and wood mice together into one index representing small mammal abundance[1].

2.3.4. Questing *Ixodes ricinus* nymph surveys

To estimate relative questing tick density at each site and through the period of host-seeking activity, questing nymphs were collected three times a year (May, July and late August/September) for two consecutive years using a standard blanket dragging method (Falco & Fish, 1992). A white 1m x 1m square of fleece blanket material was dragged over vegetation along 10m long transects. At each site, twenty transects were randomly surveyed and separated from each other by at least 20m. The transects surveyed in May were the same that I used for estimating deer density. Nymph and adult ticks that attached on the blanket were counted, collected into Eppendorf tubes and kept at -20°C until further analysis. After carrying out the 20 transects, dragging was continued until at least 100 nymphs were collected at each site (for a more robust estimation of *B. burgdorferi* s.l. prevalence) or for a maximum of 3 hours of additional dragging.

Habitat type (coniferous, deciduous or mixed woodland) was recorded at the site level while the dominant ground vegetation was recorded for each transect. A sward stick was used to estimate vegetation height at the beginning (1m), middle (5m) and end (10m) of each transect, as vegetation height can affect dragging efficiency (Gilbert, 2010). Tick surveys were conducted between 0900 hours and 1800 hours and air temperature, time, weather as well as relative humidity were also recorded for each transect. I also obtained data on rainfall from the archives

[1] Throughout this thesis, I use the term small mammal abundance for the combination of bank vole and wood mouse abundance.

of Aberdeen airport for the study period. After downloading daily rainfall data (4 data points per day) between May 2017 and September 2019, I extracted the average rainfall in the area the day before ticks were collected.

2.3.5. DNA extraction and estimation of *B. burgdorferi* s.l. prevalence

Nymphs were extracted individually using an ammonia extraction method (Gern *et al.*, 2010). Briefly, each tick was placed in an Eppendorf tube, crushed and 100µL of 0.7M NH_4OH was added. The tube was boiled at 100°C for 15 min to release the DNA. Eppendorfs were then centrifuged and boiled again for 20min with the lids open to allow the ammonia to evaporate until 50µL was left. One negative control with reagents but no tick was included for every 39 samples. Skin biopsies collected from small mammals were extracted using the Qiagen DNeasy Blood and Tissue kit and following the manufacturer's protocol (Qiagen, Hilden, Germany).

Two PCR methods were used to detect *B. burgdorferi* s.l.: nested PCR and qPCR

Ticks collected in 2017 (n=5,005) were tested using a nested PCR protocol targeting the 5S-23S intergenic spacer region (Rijpkema *et al.*, 1995) (Appendix A, S2). For the first round, each 25µL reaction contained 10x PCR buffer, 2.5mM $MgCl_2$, 0.2mM deoxynucleotide (dNTP), 0.625U of Taq DNA polymerase, 2.5pM of SN1 and SC1 primers and 4µL of extracted DNA (Table 2.1). The program consisted of 1min at 94°C (denaturation) and then 29 rounds of temperature cycling (94°C for 30s, 52°C for 30s and 72°C for 1 min). One positive (*Borrelia lusitaniae*) and one negative control were added for every 94 samples. The second round used a 1 in 10 dilution from the products obtained after the first round and the same multimix. Primers used for the second round were 2.5pM of 23SN2 and 5SCB (Table 2.1). The program for the 2nd round consisted of 1 min at 94°C followed by 39 rounds of temperature cycling (94°C for 30s, 55°C for 30s, and 72°C for 1 min). 5µL of the second-round products was then analysed on 2% agarose gel containing 2µL of ethidium bromide in Tris-borate-EDTA buffer. The PCR product from positive samples from the 2nd round of the nested PCR were sent to Edinburgh Genomics for Sanger sequencing to determine the genospecies.

B. burgdorferi s.l. was detected from samples collected in 2018 and 2019 (Appendix A, S2) using a qPCR protocol on fragments of OspA genes (Table 2.1) based on the protocol described by Heylen *et al.*, (2013). I decided to switch protocols after 2017 because I had issues with results from the nested PCR protocol (smeared bands) and I could not be sure that negative samples were not false negatives (see Appendix A, S2 for more details). The real time PCR was implemented using the IQ™ Supermix (Bio-Rad Laboratories, Hercules, USA) in a Stratagene Mx3005P thermal cycler (Agilent, Santa Clara, US). Each reaction of 20µL contained 10µL of IQ™ Supermix, primers B-OspA_modF and B-OspA_borAS, both at 200nM (Table 2.1), probe B-OspA_mod-probe-FAM at 100nM and 3µL of DNA. The qPCR program consisted of an initial activation at 95°C for 5 min followed by 60 rounds of temperature cycling (95°C for 5s followed by an annealing-extension step of 2.2°Cs^{-1} with a single point of measurement at 60°C and a cooling cycle of 37°C for 20s 52°C for 30s). One positive and one negative control were added for every 94 samples. Samples which passed the detection threshold between 40 and 60 cycles were re-tested.

Table 2.1: Names and sequences of primers and probe used in the nested and qPCR protocol.

Protocol		Designation	Sequence
Nested PCR[a]	1st round	23SN1	5'-ACCATAGACTCTTATTACTTTGAC
		23SC1	5'-TAAGCTGACTAATACTAATTACCC
	2nd round	23SN2	5'ACCATAGACTCTTATTACTTTGACCA
		5SCB	5'-biotin-GAGAGTAGGTTATTGCCAGGG
qPCR[b]		B-OspA_modF	AATATTTATTGGGAATAGGTCTAA
		B-OspA_borAS	CTTTGTCTTTTTCTTTRCTTACAAG
		B-OspA_mod-probe-FAM	FAM-AAGCAAAATGTTAGCAGCCTTGA-BHQ-1™

[a] method from Rijpkema *et al.*, 1995. [b] method from Heylen *et al.*, 2013.

Positive samples were then tested using the nested PCR protocol described above (Rijpkema *et al.*, 1995) and products from the second round were sent to Edinburgh genomics for Sanger sequencing. Samples were separated from another with a negative control to avoid contamination. Sequences were uploaded on Geneious software version 7 (https://www.geneious.com). I trimmed sequences before aligning them and I used the BLAST® data base from the National Center for Biotechnology Information (https://blast.ncbi.nlm.nig.go) to search for correspondence. A genospecies was assigned to a sample if it had more than 97%

similarity with the sequence. If the results were inconclusive or if several genospecies were detected, I sent the samples to be sequenced a second time.

Some samples tested positive with the qPCR but could not be confirmed with the nested PCR protocol. These samples were tested a second time using the nested PCR protocol and, if they were still negative, they were re-tested with the qPCR protocol to ensure they were not false positive. If they appeared positive a second time with the qPCR protocol, they were tested a third time using the nested PCR. If the sample tested negative with the nested PCR but tested positive twice with the qPCR, it was considered a positive sample. Genospecies could not be identified for these samples and they were therefore excluded from the analyses involving specific genospecies.

Because of the one-year lag between small mammal trapping and prevalence estimates in questing nymphs, prevalence estimates from samples collected in 2017 were not used in statistical analyses (as I did not have host abundance estimates for 2016) and the prevalence estimates data used for analyses were all obtained from samples tested using the qPCR protocol.

2.3.6. Statistical analyses

All statistical analyses were performed in the software R version 3.5.1 (R core team, 2013) using the glmmTMB (Brooks *et al.*, 2017; Magnusson *et al.*, 2019), lme4 (Bates, 2010) and MuMIn (Barton, 2019) packages.

I used a similar approach for all the analyses using generalized linear models (GLMs) and generalized linear mixed effect models (GLMMs). For each separate model, I first identified the appropriate distribution for the response variable. I then tested for collinearity between explanatory variables by calculating variance inflation factors (VIFs) and variables for which the VIF was above 4 were discarded from the model (Zuur *et al.*, 2009). Then, I used an F-test to assess whether continuous variables should be left in their linear forms or if some polynomial relationships with the response variable could be identified. Each continuous variable was tested in both forms (linear and polynomial) against the response variable and model fit was assessed.

Overdispersion of the data was assessed by looking at the ratio between the residual deviance and the residual degrees of freedom (Harrison, 2014). If the ratio was above 1, it indicated overdispersion and I added an observation level random effect for models using a Poisson (Harrison, 2014) and Binomial (Harrison, 2015) distributions.

If the response variable was a count, I used a GLMM with a Poisson distribution and I also fitted zero-inflation and hurdle models with a Poisson distribution, as the number of zeros might have been underestimated, and chose the most adequate model based on AICc.

Model simplification was done by either using a backward stepwise regression until only significant terms were left or using the dredge function from the MuMIn package (Barton, 2019) to test all variable combinations possible, choosing the model with the lowest AICc (Brewer *et al.*, 2016; Burnham & Anderson, 2004; Johnson & Omland, 2004; Zuur *et al.*, 2009). I used the backward stepwise regression when the model had too many parameters to run the dredge function. I used the r.squaredGLMM function from MuMIn package to calculate the conditional R^2 value, which is defined as the proportion of the total variation explained by the model (Johnson, 2014; Nakagawa & Schielzeth, 2013). The function cannot yet handle zero-inflation and hurdle GLMMs so R^2 was calculated only for models ran in lme4. When relevant, I used Post hoc comparisons using the Tukey HSD test with the Holm-Bonferroni correction to assess pairwise differentiation among levels of categorical variables.

Because roe and red deer might have different roles in feeding ticks due to differences in their ecology (e.g. home range size, social behaviour, habitat use), they were used as two separate explanatory variables in the models. While initial models aimed to have both roe and red deer density in the same analysis, this was not possible due to the complexity of the models as too many interaction terms would have been needed to account for the correlation between roe and red deer density (see section 2.4.6). Instead, I decided to enter roe and red deer separately into each model, instead of simultaneously.

2.3.6.1. Factors explaining tick abundance in the environment (DON) and on small mammal hosts

DON (i.e. number of questing nymphs counted per 10m transect) was modelled using a GLMM with a Poisson distribution as a function of the explanatory variables reported in Table 2.2. After performing F-tests, I found that temperature was a better fit in its quadratic form ($F_{1,3180}$=53.3, p<0.001) while red and roe deer abundance, vegetation height and rainfall the previous day were kept in their linear forms (Table 2.2). I added an interaction between deer density and habitat and between deer density and ground vegetation, as forest type might affect how deer use their habitat and thus their effects on DON. Relative humidity was correlated with temperature and season (VIF=4.5) and so, was not included. Random effect of site and an observation level random effect were included and continuous variables were scaled to help with convergence. I chose to ignore the one-year lag between larvae feeding on deer ($year_{t-1}$) and questing nymphs ($year_t$) because deer density remained stable across years (Appendix A, S3). Thus, I decided to use their density the same year ticks were collected so I could use the entire data set. Model simplification was done using a backward stepwise regression.

Table 2.2: Fixed and random covariates included in the full model to explain variations in questing nymph density and whether they were obtained for site or drag level. Variables in bold were retained in their quadratic forms.

	Fixed covariates	Random effects
Variables at site level	Year (2017, 2018, 2019)	Site name
	Season (May, July, September)	Observation level
	Habitat (coniferous, deciduous, mixed)	
	Rainfall the previous day	
	Roe/red deer density	
	Roe/red deer * Habitat	
	Roe/red deer * Ground vegetation	
Variables at transect level	Vegetation height	
	Ground vegetation (EV, Grass, Moss, BF)	
	Ground wetness (dry or wet)	
	Temperature	

Larval burden on small mammals was modelled for each species (bank voles and wood mice) using a GLMM with a Poisson distribution. The full models included sex, age, weight, the density of questing nymphs and the abundance of bank voles and wood mice as fixed explanatory variables and the study site and random effect of site and an observation level random effect were included. To investigate which

small mammal species was more important in feeding ticks, I also compared the average larval burden between bank voles and wood mice using a non-parametric Mann-Whitney test because the two samples were independent and not normally distributed.

2.3.6.2. The effects of deer and small mammal abundance on the prevalence of *B. burgdorferi* s.l. (NIP)

For the prevalence of *B. burgdorferi* s.l. in questing nymphs, I ran two analyses: in the first one, I investigated the effects of deer density the previous year on NIP for *B. burgdorferi* s.l. (i.e. all the genospecies combined), as it represents Lyme disease hazard for humans. In a second analysis, I focussed on NIP for *B. afzelii*, as I predicted that NIP should be affected by both deer and small mammal abundance the previous year and I wanted to investigate their effects on NIP when considered together.

NIP for *B. burgdorferi* s.l. was modelled using a binomial GLMM with a logit-link. The response variable was the proportion of ticks infected at the site level (three prevalence estimates per site and per year) and the full models included the density of red or roe deer the previous year, habitat type, the interaction between deer density and habitat, year and season (May, July or September) as fixed terms. An observation level random effect was included and the dredge function was used for model selection. As no ticks were collected in the two exclosures (sites GLS and GSB), there was not prevalence estimate so they could not be included in this analysis.

In the same manner as described for *B. burgdorferi* s.l. prevalence, I used a binomial GLMM with a logit-link to investigate whether NIP for *B. afzelii* was influenced by season, habitat, prevalence of *B. garinii* and *B. valaisiana*, to account for competition between strains, deer density the previous year (one model per deer species), year, small mammal abundance the year before and the interaction between deer and small mammal abundance, as I expected the relationship between deer and NIP to depend on small mammal abundance. I did not add an interaction term between deer density and habitat as the model would have been overparametrized. I used the dredge function to test all model

combinations possible and, because I was interested in assessing the impacts of deer and small mammals on Lyme disease hazard when considered together, I decided to present results from models that included the interaction between deer and small mammal abundance, even if they were not the best models based on AICc.

2.3.6.3. The effects of deer and small mammal abundance on density of infected nymphs (DIN) with *B. burgdorferi* s.l.

In the same manner as described for NIP, I conducted two separate analyses for DIN: in the first one, I investigated the effects of deer density on DIN for *B. burgdorferi* s.l. while in the second, I focussed the analysis on *B. afzelii*.

DIN for *B. burgdorferi* s.l. was modelled using a zero-inflation GLMM with a Poisson distribution and an offset for the area, to transform the response to integers (i.e. if 6.5 nymphs were infected/1000m^{-2}, it was transformed as 7 nymphs infected/1076m^{-2}, see Zuur *et al.*, 2009). Two models were built (one per deer species) and the full models included the density of deer the previous year, season, habitat type, temperature and vegetation height as fixed terms. Random effect of site and an observation level random effect were included. I used the dredge function for model selection. As described above, I did not include an interaction between deer density and habitat as the models would have been overparametrized.

DIN for *B. afzelii* was also modelled using a zero-inflation GLMM with a Poisson distribution and an offset for the area (Zuur *et al.*, 2009) to model. The full models included deer density the previous year (one model per deer species), season, habitat type, temperature, vegetation height, year, small mammal abundance index the previous year and the interaction between deer and small mammal abundance, as fixed effects. Random effect of site and an observation level random effect were included. I used the dredge function to test all possible models and, as previously described, I kept the best model that included the interaction between deer and small mammal.

2.3.6.4. Factors influencing small mammal and deer density

What influences small mammal abundance

Small mammal abundance (based on trapping data) was modelled using a GLMM with a Poisson distribution and an offset for the number of trap nights (i.e. if 6.6 small mammals/100TN, it was adjusted to 7/106.1TN, Zuur *et al.*, 2009). I could not use the combined index based on trapping data and vole signs as I could not use a categorical variable as my response. The response variable was the mean number of individual small mammals captured per 100 trap nights at each site and did not include recaptures. I ran one model per species of deer and the full models included deer density the same year (no data available for the previous year), habitat type, ground vegetation and rainfall the day before trapping took place, as it could affect how well traps worked, as fixed effects and site as a random effect. For the ground vegetation, I used data from the tick survey: if all the transects surveyed had the same dominant ground vegetation, I used that for the site level while, if transects were a mixed between different types of vegetation, I added a mixed vegetation category. I used the dredge function for model selection.

What influences deer density

Spatial data were obtained from Geofabrik (data source: OpenStreetMap) to assess whether some features in the environment affected deer distribution. For each site, I extracted the type of land management (i.e. hunting estate, nature reserve, forestry commission or private property), the distance to the nearest primary road and the density of tracks within the study area with a 500m buffer around the central point of each site. I used a GLMM with a Poisson distribution for roe deer and a GLM with a Poisson distribution for red deer. The response variables were the density of roe and red deer per site (number of deer/km^{-2}) with an offset for the area (Zuur *et al.*, 2009). I added the density of the other deer species as a covariate to account for potential interspecific competition and the interaction between type of land and deer density (Table 2.3), as some types of land management (e.g. hunting estates) might influence deer density. There was a correlation between the type of land and habitat (coniferous, deciduous or mixed

forest) (VIF=7) so habitat was not included in the models because I was interested in the effect of anthropogenic pressure (including hunting) on deer density. One site (DNR) was fenced in a way that would exclude red deer but would allow access to roe deer and so was removed from the analysis that focussed on red deer. I found that a quadratic relationship between the response variable and the density of the other species of deer was a better fit than linear (roe deer: $F_{1,23}$=15.8, p<0.001; red deer: $F_{1,23}$=268.8, P<0.001) and I used the dredge function for model simplification.

Table 2.3: Fixed and random terms included in full models for red and roe deer. Variables in bold were kept in their quadratic forms.

Response variable	Explanatory variables for full model	Random effect(s)
Roe deer	Density of tracks, land management type, distance to the nearest road, **density of red deer, density of red deer** * land management	Site
Red deer	Density of tracks, land management type, distance to the nearest road, **density of roe deer, density of roe deer** * land management	No random effect

2.4. Results

The density of roe deer ranged from 0 to 26.1 roe deer/km^{-2} (Figure 2.3) while red deer density ranged from 0 to 24.5 red deer/km^{-2} (Figure 2.4). A summary table summarizing the habitat type, roe and red deer density and small mammal abundance for each site is available in Appendix A, S1.

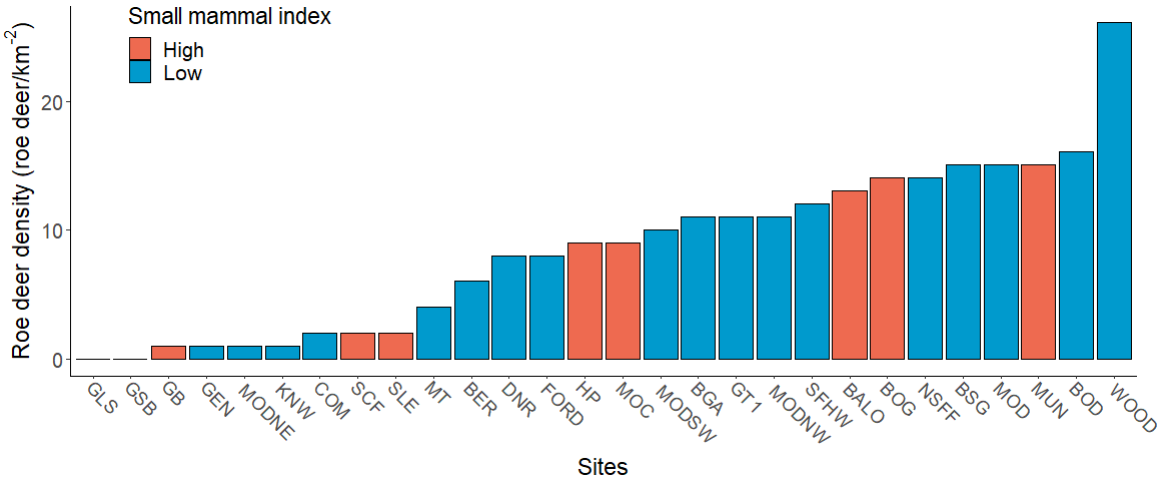


Figure 2.3: Histogram showing the density of roe deer for each site. Bars in red represent sites with high small mammal abundance index (combining trapping data and vole signs) and bars in blue represent sites with low small mammal abundance index.

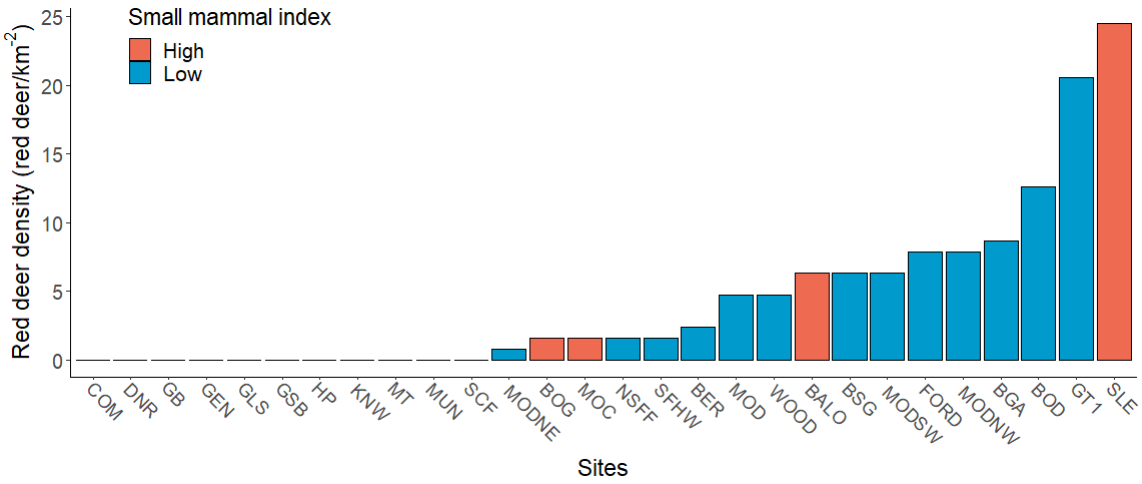


Figure 2.4: Histogram showing the density of red deer for each site. Bars in red represent sites with high small mammal abundance index (combining trapping data and vole signs) and bars in blue represent sites with low small mammal abundance index.

2.4.1. Factors explaining tick abundance in the environment (DON) and on small mammal hosts

Factors explaining tick abundance in the environment (DON)

A total of 10,168 questing nymphs were collected along transects between 2017 and 2019 (n=3,183 transects surveyed) and, on average, 3.19 nymphs were collected per $10m^{-2}$ (range: 0 to 49, SD=4.4). A random subset of 2,500 nymphs was examined under the microscope for species identification using specific keys (Marquez *et al.*, 1992) and they were all identified as *Ixodes ricinus*. Thus, it was assumed that all the ticks collected from blanket dragging were *I. ricinus*. The simplified models explaining nymph abundance can be found in Table 2.4.

Table 2.4: Fixed and random terms included in the simplified and conditional R^2 (for the full model) for red and roe deer to explain DON.

Deer species	Explanatory variables retained after model selection	Random variables	R^2
Roe deer	Year, season, habitat, rainfall the previous day, roe deer density, temperature^2, vegetation height, ground vegetation, ground wetness, roe deer density * habitat	Site name, Observation level random effect	59%
Red deer	Year, season, rainfall the previous day, red deer density, temperature^2, vegetation height, ground vegetation, ground wetness	Site name, Observation level random effect	55%

Because both models have a lot of parameters, the full summary tables are available in the supplementary material (Appendix A, S5) and Table 2.5 shows the contribution of each variable in the models. There was a positive correlation between roe deer density and DON in deciduous woodland (Figure 2.5) but no relationship in mixed and deciduous woodlands. I found a positive correlation between red deer density and DON in all woodland types (Figure 2.6).

DON was also influenced by the type of ground vegetation and fewer ticks were collected when the ground was wet (Appendix 2, S5). DON was higher in September (predicted DON=3.6, 95%CI: 1.6-9.6) compared to May (predicted DON=2.6, 95%CI: 1.1-6.8) and July (predicted DON=2.1, 95%CI: 0.9-5.5) and fewer nymphs were collected in 2017 (predicted DON=1.9, 95%CI: 0.8-4.9) compared to 2018 (predicted DON=3.2, 95%CI: 1.4-8.4) and 2019 (predicted DON=3.3, 95%CI: 1.4-8.6). DON was negatively correlated with temperature at the time of survey, ground vegetation height and rainfall the previous day (Appendix 2, S5).

Table 2.5: Summary table from the simplified models showing which variables influenced DON in the models focussing on roe (left hand side) and red deer (right hand side). ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

Roe deer				Red deer			
Variable	ΔAICc	Δlog-Lik	Δ df	Variable	ΔAICc	Δlog-Lik	Δ df
Temperature	30.1	16.1	2	Temperature	31.0	17.5	2
$Temperature^2$	23.7	11.9	1	$Temperature^2$	26.1	14.1	1
Ground vegetation	37.6	21.8	3	Ground vegetation	35.3	20.6	3
Ground wet	121.0	61.5	1	Ground wet	126.3	64.1	1
Season	104.0	54.0	2	Season	113.7	58.9	2
Habitat				Red deer $year_t$	9.6	5.8	1
Roe deer $year_t$				Vegetation height	14.8	8.4	1
Vegetation height	17.0	9.5	1	Year	77.4	40.7	2
Year	71.8	37.9	2	Rainfall day_{d-1}	21.7	11.8	1
Rainfall day_{d-1}	18.2	10.1	1				
Roe deer * Habitat	6.4	5.2	2				

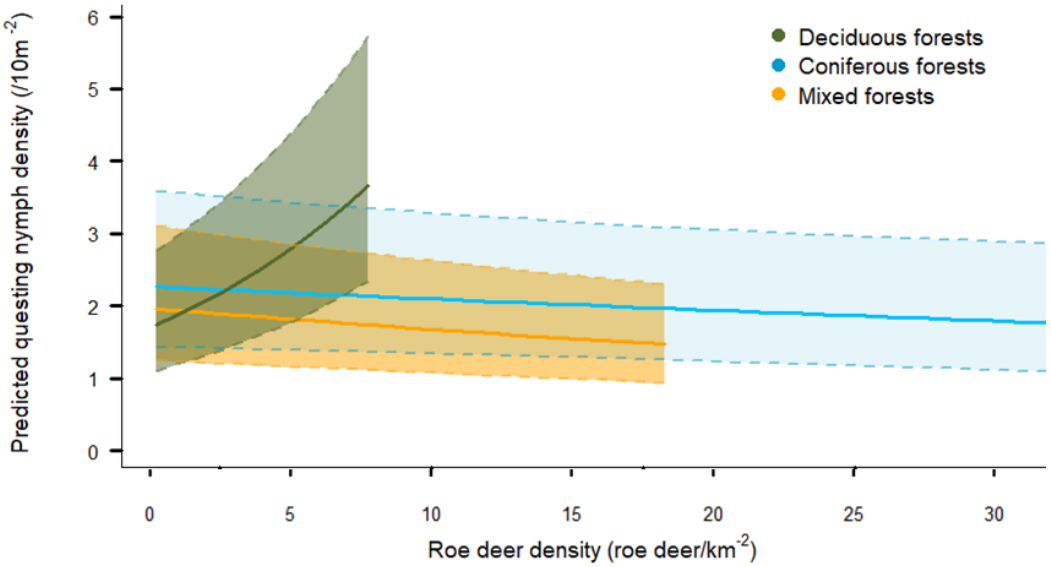


Figure 2.5: Predicted density of questing nymphs per 10m depending on roe deer density in deciduous (green), coniferous (blue) and mixed (orange) woodlands. Shaded bands represent 95%CI. The line for deciduous forests stops at 8 roe deer/km^{-2} because this was the maximum density recorded in this type of woodland. In the same manner, the line for mixed forest stops at 18/km^{-2} as it was the maximum density of roe deer recorded in this habitat.

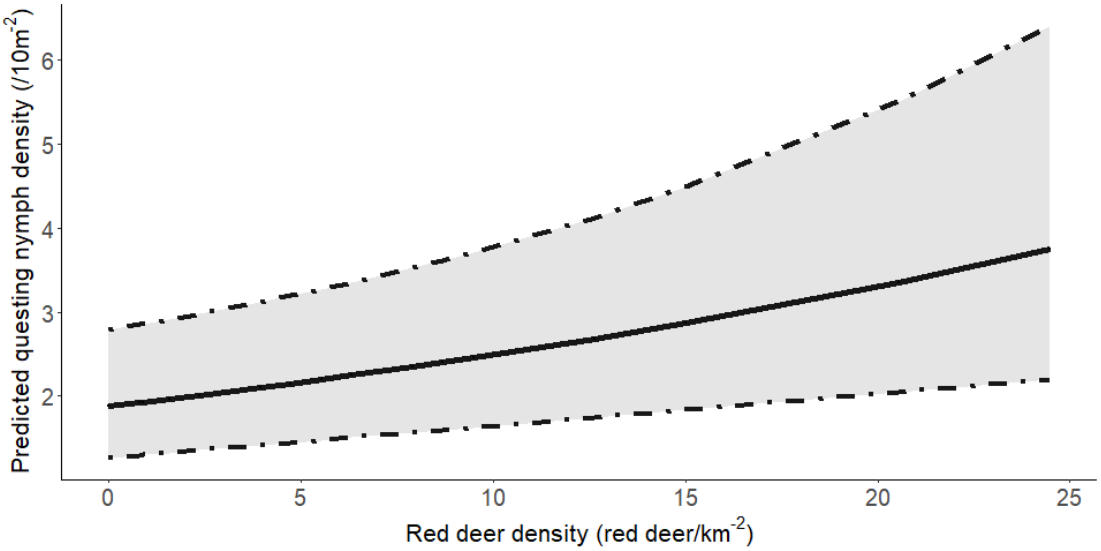


Figure 2.6: Predicted density of questing nymphs per10m depending on red deer density for all woodland types. Shaded bands represent 95%CI.

Factors explaining tick abundance on small mammal hosts

In total, 43 bank voles (22 females and 21 males) and 30 wood mice (9 females and 21 males) were captured over 4,137 trap nights in 2017 and 1,986 trap nights in 2018. On average, there were 0.07 nymphs (range: 0 to 4, SD=0.48) and 7.7 larvae (range: 0 to 41, SD=11.02) per individual small mammal. Larval burden was greater for wood mice (Median=9.0) compared to bank voles (Median=1.0) (Mann-Whitney U= 861.5, n_1=43, n_2=30, p=0.013 two tailed, Figure 2.7). As only two individuals harboured nymphs, I could not look at differences between species for nymph burdens.

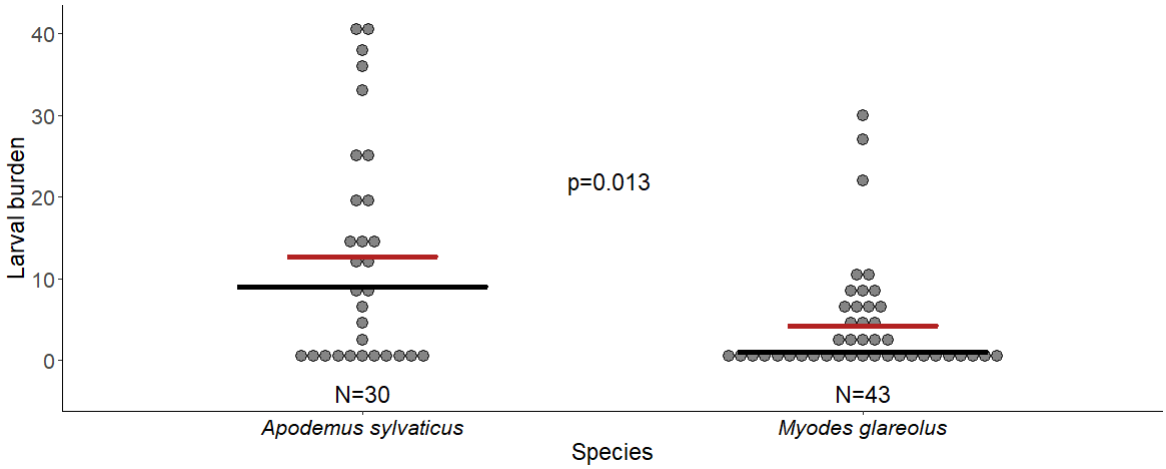


Figure 2.7: Dotplot representing larval burden counted on wood mice (*Apodemus sylvaticus*) and bank voles (*Myodes glareolus*) individuals caught during trapping. Red line represents mean and black line median. P-value from a Mann Whitney test.

Regarding larval burden on bank voles, the simplified model included questing nymph density as the only explanatory variable. Larval burden on bank voles was positively correlated with the density of questing nymphs (Table 2.6). Sex, age, abundance of small mammals and weight did not affect larval burden on bank voles. Overall, the model explained 39% of variance.

Table 2.6: Results from the selected model to explain what causes variations in larval burden for bank voles. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	-1.12	0.69			
Nymph density	0.89	0.34	4.0	3.0	1

Regarding wood mice, I found that larval burden was positively influenced by both questing nymph density and abundance of wood mice while weight, sex and age did not affect larval burden. Overall, 87% of the variance was explained by the model (Table 2.7).

Table 2.7: Results from the selected model to explain what causes variations in larval burden for wood mice. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	0.68	2.12			
Nymph density	1.25	0.49	5.5	3.8	1
Abundance of wood mice	-2.51	1.81	1.9	3.0	2
Abundance of wood mice^2	0.68	0.39	0.5	1.3	1

2.4.2. The effects of deer and small mammal abundance on the prevalence of *B. burgdorferi* s.l. (NIP)

A total of 14,775 questing nymphs were screened for *B. burgdorferi* s.l. (mean of 570 nymphs tested per site, ranging from 534 to 601, see Appendix A, S1). Out of these, 296 nymphs were found to be infected with *B. burgdorferi* s.l. (mean prevalence of 2%, 95%CI: 1.8-2.2, ranging from 0 to 14%). The predominant genospecies was *B. afzelii* (59.8% of positive nymphs, n=177) followed by *B. garinii* (16.2%, n=48), *B. valaisiana* (8.8%, n=26) and *B. burgdorferi* s.s. (8.8%,n=26) (Figure 2.8). Regarding the different PCR methods used, 5,005 nymphs were tested using the nested PCR protocol alone while 9,770 samples were tested with a qPCR (Appendix A, S2). Out of these 9,770 samples, 201 tested positive but 10.4% (n=21) could not be amplified using the nested PCR and thus, could not be sequenced for genospecies identification. As they appeared positive twice on the qPCR, they were considered as true positives and classified as *B. burgdorferi* s.l. They were discarded for the analysis focussing on specific genospecies but were included to investigate the effect of deer on *B. burgdorferi* s.l.

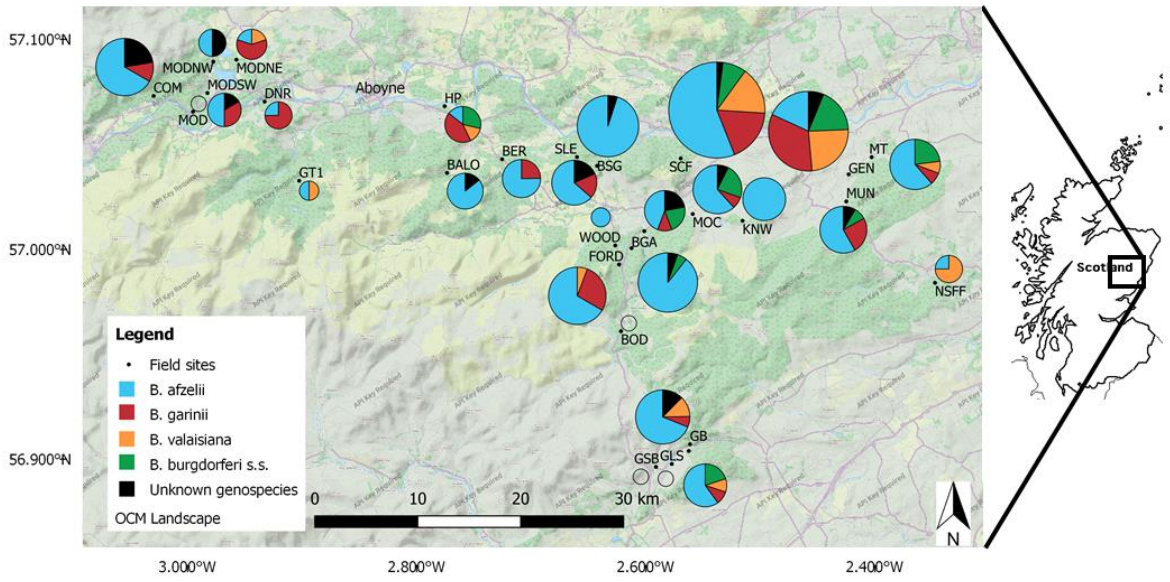


Figure 2.8: Map representing the 28 study sites surveyed and the prevalence of *B. burgdorferi* s.l. in questing I. ricinus nymphs. Transparent circles represent sites for which prevalence of *B. burgdorferi* s.l. was 0% (MOD, BOD, GSB, GLS). the size of pie chart is proportional to nymphal infection prevalence and the various colours correspond to the proportion of each genospecies found. Black represents samples which were positive on the qPCR but could not be sequenced.

Seventy-seven ear biopsies (46 from bank voles and 31 from wood mice) were tested. However, none was found to be positive for *B. burgdorferi* s.l.

Prevalence of *B. burgdorferi* s.l.

When I investigated the effects of roe deer on NIP for *B. burgdorferi* s.l., the simplified model included habitat and roe deer density the previous year and I found a strong, negative association between NIP and roe deer density (Table 2.8 & Figure 2.9). NIP was higher in coniferous woodlands (predicted NIP=1.8, 95%CI: 1.1-2.8) compared to deciduous woodlands (predicted NIP=0.4, 95%CI: 0.1-1.0) but similar to mixed forests (predicted NIP=0.9, 95%CI: 0.4-1.9) (Table 2.8 and Appendix 2, S6) while year and season did not affect NIP. Overall, the model focussing on roe deer explained 26% of the variance.

Table 2.8: Results from the selected model to explain what causes variations in *B. burgdorferi* s.l. prevalence when including roe deer. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	-2.77	0.28			
Roe deer density year$_{t-1}$	-0.12	0.03	14.6	8.3	1
Habitat			7.5	5.7	2
Deciduous	-1.62	0.58			
Mixed	-0.71	0.38			

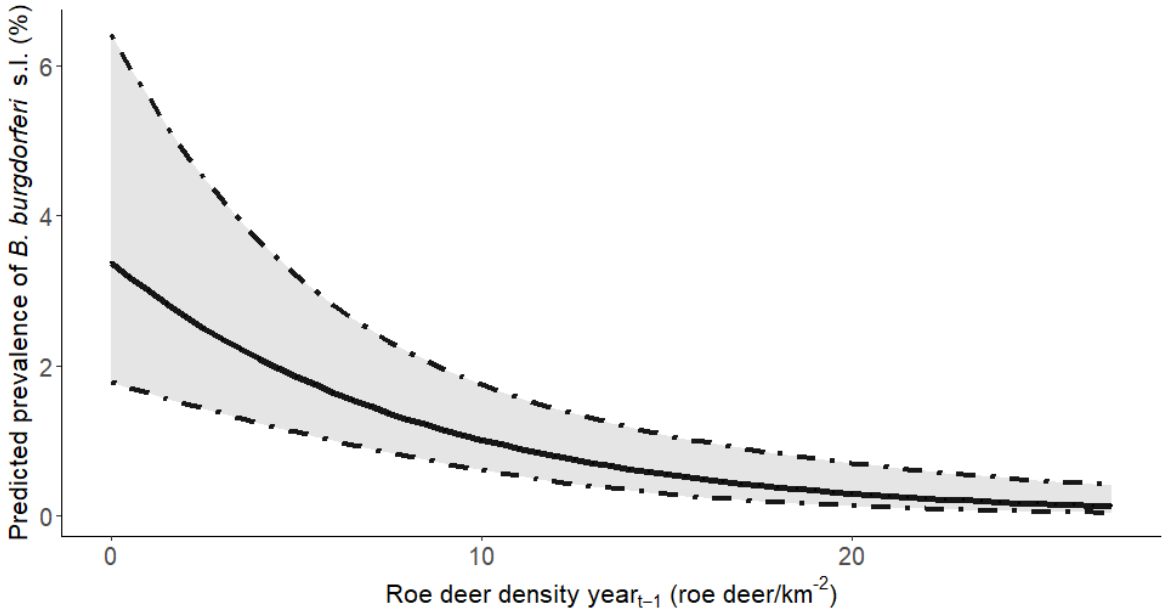


Figure 2.9: Predicted prevalence of *B. burgdorferi* s.l. in questing nymphs depending on the mean density of roe deer the previous year. Shaded bands represent 95%CI.

In the analysis focussing on the role of red deer as a potential dilution host, the simplified model included season as the only explanatory variable and I found no association between NIP for *B. burgdorferi* s.l. in questing ticks and the density of red deer the previous year (Table 2.9).

Table 2.9: Selection of models of *B. burgdorferi* s.l. prevalence in questing ticks depending on red deer density: This table shows the first five models obtained with the dredge function along with their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the one selected and did not include red deer density the previous year. Hab: habitat.

Red deer $year_{t-1}$	Red deer $year_{t-1}$ * Hab	Hab	Season	Year	df	Log-Lik	AICc	ΔAICc
-	-	-	-	+	4	-139.2	287.0	0
-	-	-	+	+	6	-137.0	287.3	0.3
-	-	-	-	-	3	-140.6	287.5	0.5
-	-	-	+	-	5	-138.3	287.6	0.6
-	-	+	-	-	5	-138.4	287.7	0.7

Prevalence of *B. afzelii*

Because I was interested in assessing the impacts of deer and small mammals on Lyme disease hazard when considered together, I presented the results from the best models that included the interaction between deer and small mammal abundance (Table 2.10 & Table 2.11).

Table 2.10: Selection of models of the effects of roe deer on NIP with *B. afzelii*: table showing the first six models obtained with the dredge function along with their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The best model containing both deer and small mammals as predictors (shaded in grey) was the only I chose to report results from as I was interested in the effects of deer and small mammals when considered together. Vege: vegetation height, Hab: habitat type, B.g: *B. garinii* and B.v: *B. valaisiana*.

Roe deer	Season	Hab	Rodent	Rodent * deer	NIP B.g	NIP B.v	Year	df	log-Lik	AICc	ΔAICc
+	+	+	-	-	-	-	-	8	-95.4	209.1	0
+	+	-	-	-	-	-	+	7	-97.0	209.8	0.8
+	-	+	-	-	-	-	+	9	-98.5	209.8	0.8
+	+	+	-	-	-	-	-	6	-95.0	210.3	1.2
+	-	+	-	-	+	-	-	9	-97.6	210.9	1.8
+	+	+	+	+	-	-	-	10	-94.7	213.1	4.0

Table 2.11: Selection of models of the effects of red deer on NIP with *B. afzelii*: table showing the first six models obtained with the dredge function along with their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The best model containing both deer and small mammals as predictors (shaded in grey) was the only I chose to report results from as I was interested in the effects of deer and small mammals when considered together. Vege: vegetation height, Hab: habitat type, B.g: *B. garinii* and B. v: *B. valaisiana*.

Red deer	Season	Hab	Rodent	Rodent * deer	NIP B.g	NIP B.v	Year	df	log-Lik	AICc	ΔAICc
-	+	-	-	-	-	-	+	6	-99.1	211.4	0.0
+	+	-	-	-	-	-	-	5	-99.1	212.1	0.7
+	+	-	-	-	-	+	+	6	-98.2	214.3	2.9
-	+	-	+	-	-	-	-	7	-100.0	214.4	3.1
-	+	-	-	-	+	-	-	7	-100.1	214.6	3.2
-	-	+	-	-	-	-	+	4	-100.3	214.7	3.4
+	-	+	-	-	-	-	-	6	-100.1	214.8	3.4
+	+	-	+	+	-	-	-	8	-98.4	214.9	3.5

When investigating how a combination of small mammal and roe deer density the previous year affected NIP for *B. afzelii*, the simplified model included season, habitat and the interaction between small mammal and roe deer and it explained 29% of the variance. NIP was negatively associated with roe deer density and this negative correlation was stronger in sites with high small mammal abundance (Table 2.12 & Figure 2.10-A).

For the model focussing on red deer, year, season and the interaction between red deer and small mammal abundance were retained in the model and it explained 26% of the variance. Results showed increased NIP with red deer density for sites with high small mammal abundance while NIP for sites with low small mammal abundance showed no relationship with red deer density (Table 2.12 & Figure 2.10-B).

NIP for *B. afzelii* was higher in September (predicted NIP=0.9, 95%CI: 0.3-2.5) compared to July (predicted NIP=0.4, 95%CI: 0.1-1.2) and it was higher in coniferous woodlands (predicted NIP=1.1, 95%CI: 0.5-2.6) compared to deciduous ones (predicted NIP=0.2, 95%CI: 0.03-0.9) (Appendix 2, S7).

Table 2.12: Results from the selected models (roe and red deer) to explain what causes variations in *B. afzelii* prevalence. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

Variable	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Roe deer model					
Intercept	-4.05	0.63			
Roe deer density year$_{t-1}$	-0.13	0.06			
Season			2.6	2.3	2
May	0.18	0.40			
September	0.86	0.37			
Habitat			3.0	2.5	2
Deciduous	-1.86	0.79			
Mixed	-0.88	0.54			
Small mammal index: low	-0.13	0.74			
Roe deer density year$_{t-1}$*small mammal: low	0.06	0.07	-2.6	-0.3	1
Red deer model					
Intercept	-5.89	0.50			
Red deer density year$_{t-1}$	0.09	0.04			
Season			3.7	3.9	2
May	0.17	0.41			
September	0.88	0.37			
Small mammal index: low	0.69	0.53			
Red deer density year$_{t-1}$*small mammal: low	-0.09	0.06	-0.5	0.7	1

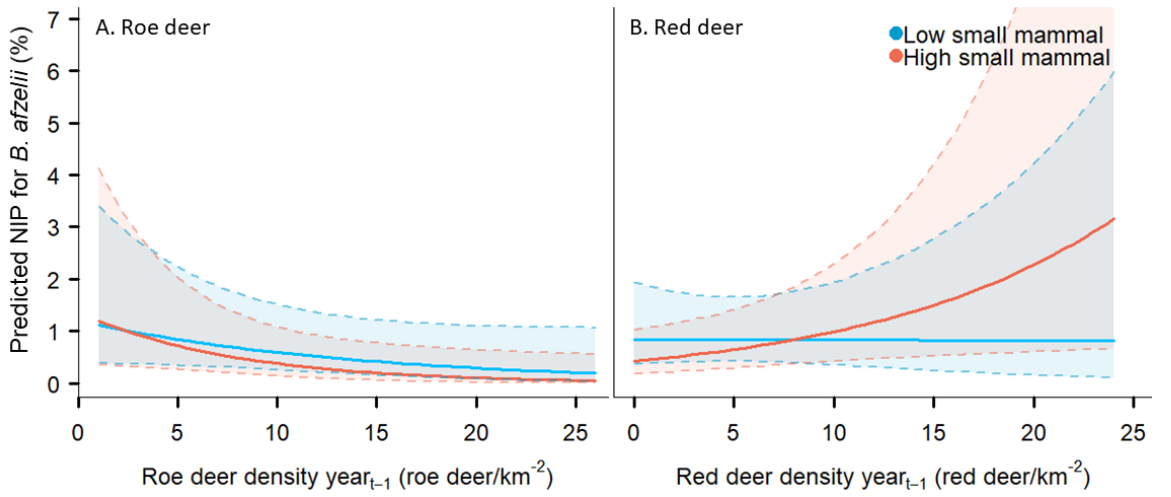


Figure 2.10: Predicted nymphal infection prevalence (NIP) for *B. afzelii* depending on roe (A) and red (B) deer density the previous year in site with high (red) and low (blue) small mammal abundance the previous year. Shaded bands represent 95%CI.

2.4.3. The effects of deer and small mammal abundance on the density of infected nymphs (DIN) with *B. burgdorferi* s.l.

Density of questing nymphs infected with *B. burgdorferi* s.l.

For the analysis focussing on the role of roe deer, the simplified model included only temperature and habitat as explanatory variables and I found no association between DIN with *B. burgdorferi* s.l. and the density of roe deer the previous year (Table 2.13).

Table 2.13 Selection of models of the effects of roe deer on DIN with *B. burgdorferi* s.l.: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the one selected and did not include roe deer density the previous year. Temp: temperature, vege: vegetation and hab: habitat.

Roe deer	Season	Hab	Temp	Vege height	Year	df	Log-Lik	AICc	ΔAICc
-	-	+	+	-	-	6	-159.7	331.4	0
-	-	+	+	-	+	7	-159.6	333.2	1.8
-	-	+	+	+	-	7	-159.7	333.3	1.8
-	+	+	+	-	-	8	-159.1	334.1	2.7

Similarly to the model focussing on roe deer, the simplified model also included only temperature and habitat and there was no association between red deer density and DIN (Table 2.14).

Table 2.14: Selection of models of the effects of red deer on DIN with *B. burgdorferi* s.l.: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the one selected and did not include red deer density the previous year. Temp: temperature, vege: vegetation and hab: habitat.

Red deer	Season	Hab	Temp	Vege height	Year	df	Log-Lik	AICc	ΔAICc
-	-	+	+	-	-	6	-159.7	331.4	0
+	-	+	+	-	-	7	-159.1	332.2	0.8
-	-	+	+	-	+	7	-159.6	333.2	1.8
-	-	+	+	+	-	7	-159.7	333.3	1.9
+	-	+	+	+	-	8	-158.9	333.8	2.4
-	+	+	+	-	-	8	-159.1	334.1	2.7
+	-	+	+	-	+	8	-159.1	334.2	2.8
+	+	+	+	+	-	10	-157.2	334.3	2.9

DIN was negatively affected by temperature (Appendix 2, S8) and more nymphs were found infected per unit of area in coniferous (predicted DIN=10.5, 95%CI: 7.9-13.9) compared to deciduous (predicted DIN=2.6, 95%CI: 1.2-5.3) and mixed forests (predicted DIN=4.7, 95%CI: 2.6-8.3) (Appendix 2, S8). Ground vegetation height, season and year did not affect DIN.

Density of nymphs infected with *B. afzelii*

Because I was interested in assessing the impacts of deer and small mammals on Lyme disease hazard when considered together, I decided to present results from the best models that included the interaction between deer and small mammal abundance (Table 2.15 & Table 2.16).

Table 2.15: Selection of models of the effects of roe deer on DIN with *B. afzelii*: Table showing the first six models obtained with the dredge function along with their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The best model containing both deer and small mammals as predictors (shaded in grey) was the only I chose to report results from as I was interested in the effects of deer and small mammals when considered together. Vege: vegetation height, Hab: habitat type.

Vege	Roe deer	Hab	Rodent	Temp	Season	Year	Rodent* deer	df	log-Lik	AICc	ΔAICc
+	-	+	-	-	+	+	-	9	-121.1	263.9	0.0
-	-	+	-	-	+	+	-	8	-122.5	263.9	0.0
-	+	+	-	-	+	+	-	9	-121.5	264.5	0.6
-	-	+	-	+	-	-	-	6	-126.1	265.8	2.0
+	-	+	-	+	-	-	-	7	-125.7	267.5	3.6
-	+	-	+	-	+	+	+	9	-123.8	269.1	5.3

Table 2.16: Selection of models of the effects of red deer on DIN with *B. afzelii*: Table showing the first six models obtained with the dredge function along with their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The best model containing both deer and small mammals as predictors (shaded in grey) was the only I chose to report results from as I was interested in the effects of deer and small mammals when considered together. Vege: vegetation height, Hab: habitat type.

Vege	Red deer	Hab	Rodent	Temp	Season	Year	Rodent* deer	df	log-Lik	AICc	ΔAICc
-	+	-	+	-	+	+	+	9	-118.0	257.7	0.0
-	+	+	+	-	+	+	+	11	-115.2	258.0	0.3
-	+	+	+	-	+	-	+	10	-117.5	259.6	1.9
-	+	+	+	+	+	-	+	12	-114.5	259.6	1.9

Regarding roe deer, the simplified model included roe deer density the previous year, small mammal abundance and their interaction, season and year. DIN for *B. afzelii* was positively correlated with roe deer density the previous year, regardless of small mammal abundance (Table 2.17 & Figure 2.11-A).

Regarding red deer, the simplified model (which was the model with the lowest AICc in this case) included red deer density the previous year, small mammal abundance and their interaction, season and year. DIN for *B. afzelii* was positively correlated with red deer density the previous year and this relationship was stronger in sites having low small mammal abundance (Table 2.17 & Figure 2.11-B). DIN was higher in September (predicted DIN= 31, 95%CI: 10-99) compared to May (predicted DIN= 6.5, 95%CI: 2.6-17) and July (predicted DIN= 3.1, 95%CI: 1.1-8.5). DIN was also higher in 2019 (predicted DIN= 20, 95%CI: 6.7-64) compared to 2018 (predicted DIN= 6.6, 95%CI: 2.4-18.9) (Appendix 2, S9) while it was not affected by temperature, vegetation height or habitat type.

Table 2.17: Summary table from the conditional sections of selected models (one per deer species) showing which variables influence the density of nymphs infected with *B. afzelii*. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

Variable	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Roe deer					
Intercept	-7.81	0.62			
Roe deer density year$_{t-1}$	0.04	0.05			
Season			147.9	75.9	2
May	1.02	0.45			
September	2.41	0.58			
Year: 2019	1.85	0.49	17.1	9.5	1
Small mammal: low	-0.04	0.72			
Roe deer density year$_{t-1}$*small mammal: low	0.06	0.08	-1.5	0.2	1
Red deer					
Intercept	-7.46	0.52			
Red deer density year$_{t-1}$	0.07	0.03			
Season			10.0	7.0	2
May	0.74	0.43			
September	2.31	0.55			
Year: 2019	1.13	0.48	3.8	2.9	1
Small mammal: low	-0.20	0.48			
Red deer density year$_{t-1}$*small mammal: low	0.16	0.07	3.0	2.5	1

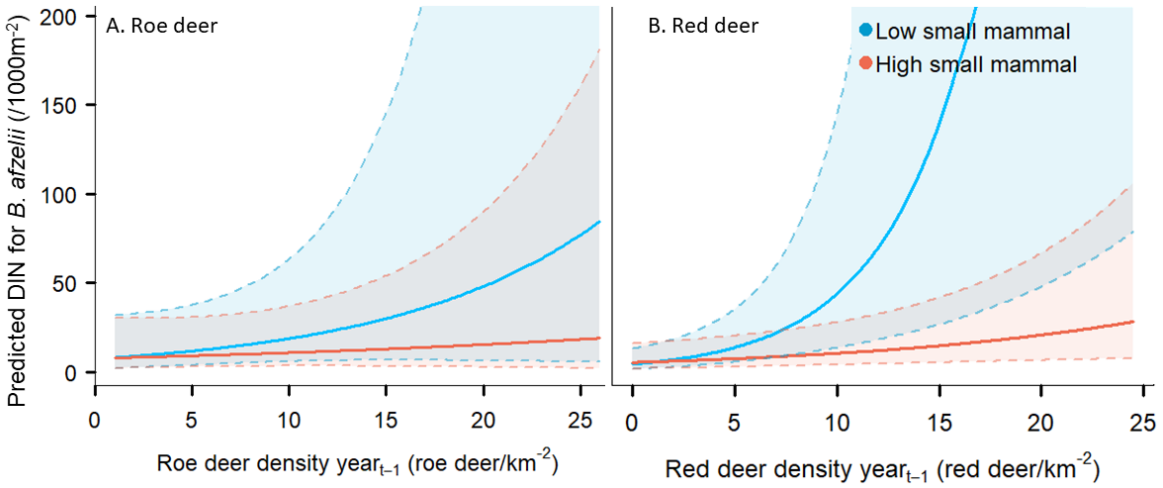


Figure 2.11:Predicted density of nymphs infected with *B. afzelii*/1000m^{-2} depending on (A) roe and (B) red deer density the previous year and in sites presenting high (red) and low (blue) small mammal abundance. Shaded bands represent 95%CI.

2.4.4. Factors influencing small mammal and deer density

Small mammal abundance

When assessing what affected small mammal abundance (trapping data), the simplified model focussing on roe deer density included ground vegetation only and small mammal abundance was not influenced by roe deer density, habitat or rainfall the previous day (Table 2.18).

Table 2.18: Table showing the first three models obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the one selected and did not include food supplementation.

Ground vegetation	Habitat	Rainfall$_{d-1}$	Roe deer year$_t$	df	log-Lik	AICc	ΔAICc
+	-	-	-	6	-56.0	128.0	0
+	+	-	-	8	-52.7	128.9	0.9
+	-	-	+	7	-56.0	131.6	3.6

For the model focussing on red deer, there was a negative correlation between small mammal abundance (trapping data) and the density of red deer (Table 2.19 & Figure 2.12). The simplified model also included ground vegetation. As for the other model, capture rates were not affected by habitat or rainfall (Table 2.19 & Appendix A, S10). Overall, the model including red deer explained 27% of the variance. Sites with a ground cover of grass had fewer small mammals (predicted

abundance: 0.2, 95%CI: 0.09-0.7) compared to site with brackens/ferns (predicted abundance: 1.2, 95%CI: 0.5-3.6), *ericaceous/vaccinium* (predicted abundance: 2.7, 95%CI: 1.4-5.9) and mixed vegetation (predicted abundance: 1.6, 95%CI: 0.6-4.3). Small mammal abundance in sites with bracken/ferns, mixed vegetation, moss (predicted abundance: 1.0, 95%CI: 0.3-3.6) and ericaceous/*vaccinium* all had similar small mammal abundance (Appendix A, S10).

Table 2.19: Results from the selected model to explain what causes variations in small mammal abundance depending on red deer density. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

Variable	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	-3.87	0.38			
Red deer density $year_t$	-0.15	0.06	7.0	4.5	1
Ground vegetation			13.4	10.7	4
EV	0.84	0.49			
Grass	-1.60	0.55			
Mixed	0.31	0.62			
Moss	-0.17	0.62			

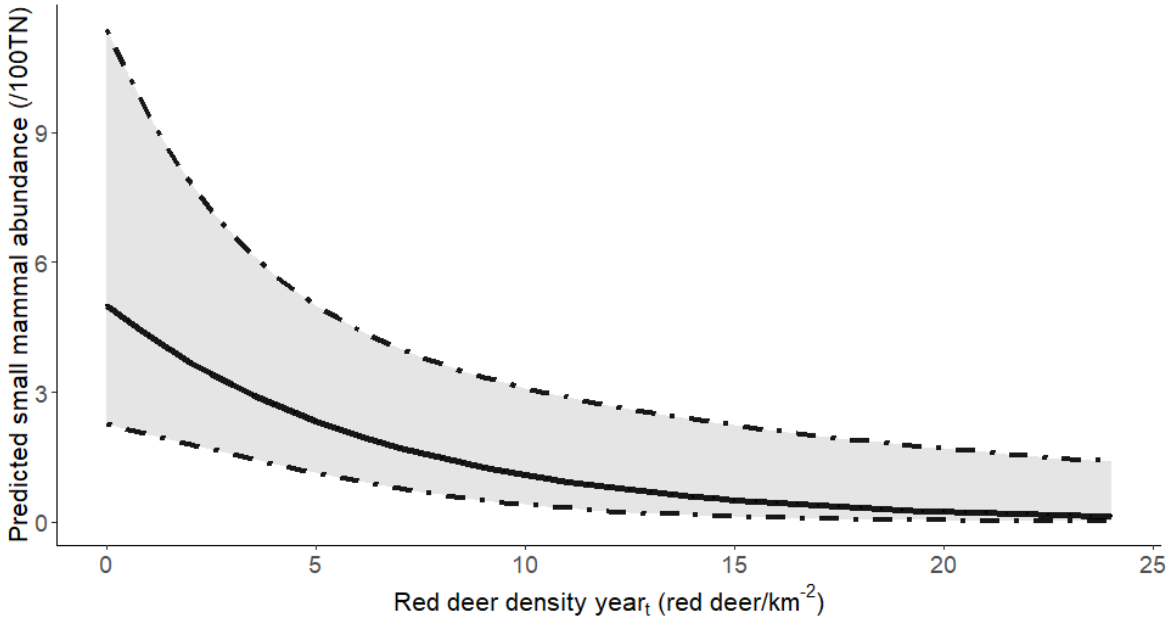


Figure 2.12: Predicted small mammal abundance/100 trap nights depending on red deer density. Shaded bands represent 95%CI.

Deer density

For this analysis, I investigated which factors correlated with deer density. For roe deer, the simplified model included the density of red deer and the density of tracks within the site with a 500m buffer. Roe deer were negatively impacted by the density of tracks while they were not affected by the distance to the nearest primary road or the type of land management (Table 2.20, Figure 2.13). There was a non-linear relationship between roe and red deer densities: roe deer density was positively correlated with red deer until red deer density reached 10 individuals/km^{-2}. However, above that, roe deer density was negatively impacted by higher red deer densities (Table 2.20, Figure 2.14-A). The model explained 56% of the variance.

Table 2.20: Results from the selected model to explain what causes variations in roe deer density. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	2.43	0.14			
Red deer density	1.08	0.21	16.7	10.4	2
Red deer density^2	-0.40	0.10	11.6	6.8	1
Track density	-0.24	0.13	1.6	1.8	1

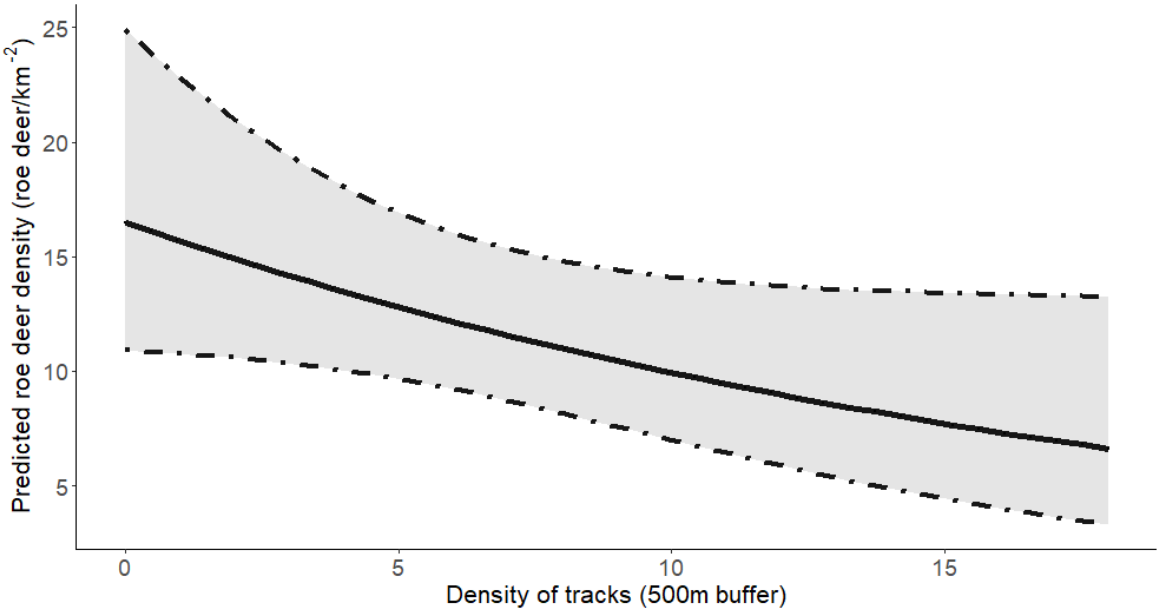


Figure 2.13: Plots presenting the predicted density of roe deer/km^{-2} depending on the density of human paths within a 500m from the site. Shaded bands represent 95%CI.

Regarding red deer, their density varied only with roe deer density and they were unaffected by the type of land management, density of human paths, the distance to the nearest primary road or the density of building in the area. Red deer density was positively correlated with roe deer until red deer density reached 16

individuals/km^{-2} above which they were negatively associated (Table 2.21 & Figure 2.14-B). The model explained 82% of the variance.

Table 2.21: Results from the selected model to explain what causes variations in red deer density. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	0.92	0.14			
Roe deer density	1.63	0.19	145.5	74.7	2
Roe deer density^2	-0.32	0.07	22.9	12.4	1

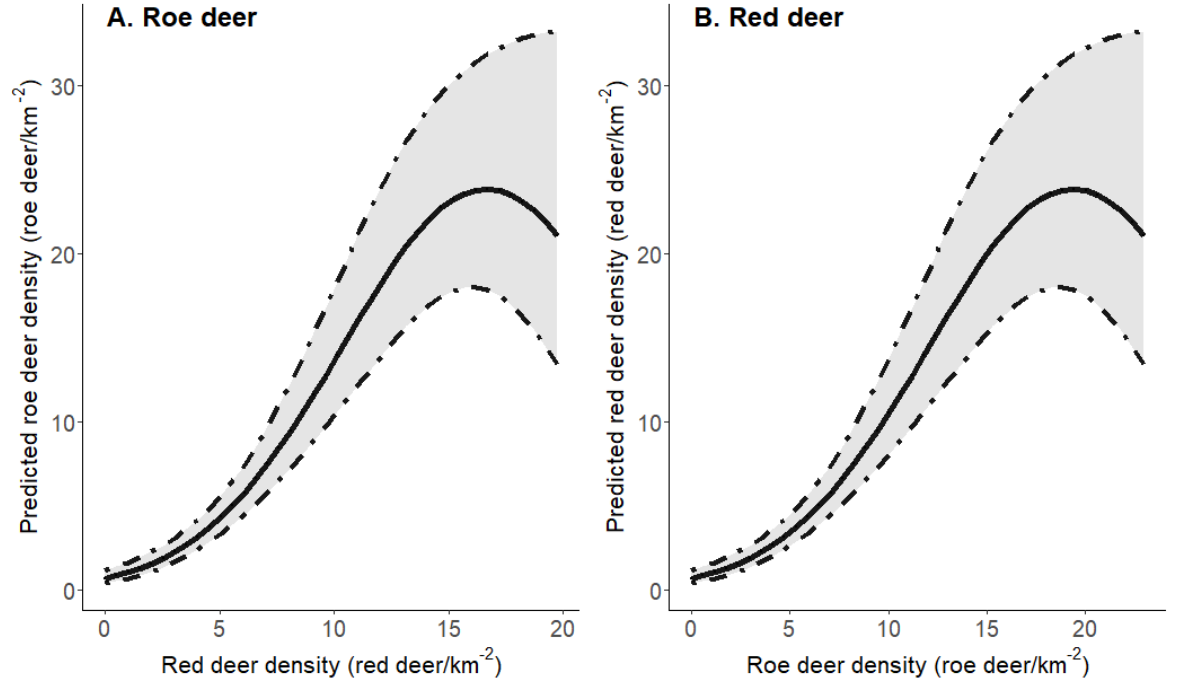


Figure 2.14: Plots presenting the predicted density of roe deer/km^{-2} depending on red deer density (A) and the predicted density of red deer/km^{-2} depending on roe deer density (B). Shaded bands represent 95%CI.

2.5. Discussion

This cross-sectional survey allowed me to empirically assess the impacts of host community composition on Lyme disease hazard, defined as the density of infected nymphs (DIN), which combines both tick abundance (DON) and *B. burgdorferi* s.l. prevalence (NIP). I observed a positive correlation between DON and both red and roe deer densities and there was a dilution effect from roe deer on NIP for both *B. burgdorferi* s.l. and *B. afzelii*, regardless of small mammal abundance. There was a strong, positive association between both roe and red deer density and DIN with *B. afzelii* and this relationship was more marked in sites having low small mammal abundance. I also identified multiple factors affecting

host abundance including interspecific interactions and anthropogenic pressure, which could impact Lyme disease hazard. Results from this study highlight the complexity of the Lyme disease epidemiological cycle and the need to decorticate the different mechanisms involved.

The main goal for this study was to identify the role of the combination of both deer (as tick reproduction hosts and possible *B. burgdorferi* s.l. dilution hosts) and small mammals (as *B. afzelii* reservoir hosts) in driving DIN through their effects on DON and NIP.

The first mechanism I investigated was the effect of deer on tick abundance. As predicted, there was a positive correlation between DON and deer density, confirming their role in driving tick populations (Gilbert *et al.*, 2012; Krasnov *et al.*, 2002; Minakawa *et al.*, 2002; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014). To further confirm that, I used two exclosures in this study and no ticks were collected during the survey in these plots, which is consistent with earlier studies (Hofmeester *et al.*, 2017b; Mysterud *et al.*, 2016), and demonstrates the key role of reproduction hosts. While there was a positive correlation between red deer density and DON in general (Figure 2.6), this was only the case in deciduous woodlands for roe deer (Figure 2.5). Several other studies have found higher tick densities in deciduous woodlands compared to coniferous forests and hypothesized that it could be due to thicker leaf litter on the ground providing adequate microclimate for ticks and denser canopy cover shielding them from wind and UV rays (Estrada-peña, 2001; Lindström & Jaenson, 2003; MacLean, 2016; Pfäffle *et al.*, 2013; Vourc'h *et al.*, 2016). Another explanation would be that deciduous woodlands harbour more small mammals (Heyman *et al.*, 2009) and thus, a higher number of larval ticks are able to find a host and successfully moult into nymphs. However, there is no evidence for that in this analysis as forest type was not a predictor of small mammal abundance (Table 2.18). This is also reflected by results for larval burden on small mammals, which was not dependent on habitat type. Nevertheless, because habitat was not retained as a predictor for the model focussing on red deer the disparity observed could be explained by differences in the ecology between roe and red deer (Latham *et al.*, 1996; Welch *et al.*, 1990). It might indicate that roe deer spend more time in deciduous forests compared to coniferous or mixed woodlands while red deer use all three types of woodland

equally (Hofmeester *et al.*, 2017b). Unfortunately, using pellet counts to estimate deer density does not provide information about how both red and roe deer are using their habitats and this is one of the caveats in Lyme disease ecology research at the moment that needs to be addressed. For instance, using GPS data from tagged individuals could provide information on habitat use.

As mentioned above, larval burden on small mammals was not related to habitat type. However, wood mice fed, on average, more larvae compared to bank voles (Figure 2.7). These results are in line with several other studies (Kiffner *et al.*, 2011b; Tälleklint & Jaenson, 1997; van Duijvendijk *et al.*, 2016b), suggesting that these two species differ in their contribution to feeding larvae (Perez *et al.*, 2016b) and, potentially, could affect DON. Bank voles are known to develop higher acquired resistance to Ixodid ticks (Dizij & Kurtenbach, 1995; Hanincová *et al.*, 2003a) and ticks feeding on bank voles have a lower probability of successfully moulting (Humair *et al.*, 1999; van Duijvendijk, 2016). Thus, it could be hypothesized that bank voles have a lesser role in the Lyme disease epidemiological cycle compared to wood mice. However, bank voles have a higher reservoir competency for *B. burgdorferi* s.l. (Kurtenbach *et al.*, 1994; Paulauskas *et al.*, 2008; Tälleklint & Jaenson, 1994) so this effect could be balanced. There was no difference in larval burden between sex for either species, which is surprising as many studies found that males harboured more ticks than females (Brunner & Ostfeld, 2008; Ostfeld *et al.*, 1996; Tälleklint & Jaenson, 1997). This might be due to the small numbers of small mammals captured, which could also explain why results showed a positive correlation between wood mice burden and wood mice density, as it goes against other findings (Levi *et al.*, 2016; Shaw *et al.*, 2003). Some studies found a correlation between burden and infection rate of individuals (Hanincová *et al.*, 2003a; Taylor *et al.*, 2013). However, no small mammals were found to be infected in this study (from ear biopsies) so I could not test this hypothesis. These findings highlight the importance of considering competent reservoir species separately as they are unlikely to contribute to the epidemiological cycle in the same way.

The second component to consider when investigating Lyme disease hazard is pathogen prevalence. I first assessed the effects of deer density the previous year on NIP for *B. burgdorferi* s.l. As predicted, there was a negative correlation

between roe deer density the previous year and NIP (Figure 2.9), providing one of a the few pieces of empirical evidence for the role of deer as dilution hosts in Europe (Rosef *et al.*, 2009; Vourc'h *et al.*, 2016). Surprisingly, I did not find an association between red deer density and NIP. Even though roe and red deer occupy different niches, I would still expect to observe a dilution effect for both species as they both carry high tick burdens (Pacilly *et al.*, 2014; Tälleklint & Jaenson, 1997; Vázquez *et al.*, 2011; Vor *et al.*, 2010). I did not take into account other hosts that could potentially be involved such as squirrels and meso-predators (e.g. foxes *Vulpes vulpes* and badgers *Meles meles*) and that could also explain the small variance explained by the models (Takumi *et al.*, 2019).

While this first analysis focussing on the role of deer as a dilution host for *B. burgdorferi* s.l. showed how roe deer could dilute pathogen prevalence, I was interested in the ratio of competent reservoir hosts (small mammals) and non-competent hosts (deer) to get a clear picture of the epidemiological cycle. Although I mentioned above that bank voles and wood mice should be considered as two different species, I did not have a sample size large enough to do so here and I combined them together. To simplify the analysis further, I grouped them into a categorical index distinguishing high and low abundance. I expected to observe a decrease in the prevalence of *B. afzelii* as a function of increasing deer densities as more ticks should feed on non-competent deer rather than competent small mammals. Similarly to the previous analysis, I found a negative correlation between roe deer and NIP for *B. afzelii*, regardless of small mammal abundance (Figure 2.10). I predicted that NIP should be correlated with small mammal abundance and the lack of a relationship that I observed might be due to the trapping method I used. Indeed, I used traps that excluded shrews, which are considered important hosts in the Lyme disease epidemiological cycle in North America (Brisson & Dykhuizen, 2004; LoGiudice *et al.*, 2003). When I investigated the effect of red deer on NIP, there was a negative correlation between red deer density and NIP in sites with low small mammal abundance (Figure 2.10). While this result is in line with my predictions of a dilution effect, I was not expecting the positive association between NIP and red deer density for sites with high small mammal abundance (Figure 2.10). Even though these results are interesting, it must be noted that the confidence intervals are very large. This increase between red deer and NIP observed might come down to the method used to quantify deer

density. Some sites had high number of deer pellets but I do not know if it was due to a few individuals spending a lot of time in this woodland or a large herd of red deer that passed very quickly to reach another patch of forest and that might not have picked up ticks.

My results showed that NIP for *B. afzelii* was higher in September compared to May and July (Coipan *et al.*, 2013; Reye *et al.*, 2010) but did not pick this difference in the previous model using *B. burgdorferi* s.l. *B. afzelii* might have a more marked seasonality because small mammal abundance also increases along the summer (Montgomery, 1989; Olsson *et al.*, 2002) so the higher NIP I observed in September might indicate larvae that fed on small mammals early in the summer and moulted in the same year.

These findings confirm the role of deer in driving vector abundance (Gilbert *et al.*, 2012; Krasnov *et al.*, 2002; Minakawa *et al.*, 2002; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014) and also point to their potential role in disease mitigating through a dilution effect on NIP (Rosef *et al.*, 2009; Vourc'h *et al.*, 2016), as it was shown for roe deer. I also investigated the role of small mammals in the epidemiological cycle and, even though I did not observe what I was expecting, there is evidence for their role in driving pathogen prevalence (Ostfeld *et al.*, 2001; Ostfeld *et al.*, 2018b; Vuong *et al.*, 2017).

In terms of Lyme disease hazard, I predicted that the lowest risk for Lyme disease should be in an ecosystem supporting low densities of small mammals and deer. When focussing the analysis on *B. afzelii*, I found evidence supporting this prediction. However, results indicated that deer density was a more limiting factor than small mammal abundance for DIN, as low DIN was associated with low densities of deer regardless of small mammal abundance (Figure 2.11). As predicted, I found a positive relationship between roe and red deer densities the previous year and DIN and these findings are in line with those of Takumi *et al.*, (2019) and Vourc'h *et al.*, (2016). For both deer species, I found a stronger, positive effect on DIN in sites having low small mammal abundance and the highest DIN was in sites with high deer density and low small mammal abundance, which goes against my predictions (Figure 2.11). When conducting the survey, I could only find one site having both high densities of deer and small mammal, probably

due to the negative effects of red deer on small mammals observed (Figure 2.12) (Flowerdew & Ellwood, 2001; Smit *et al.*, 2011; van Wieren & Bakker, 2008). These results imply that the highest risk for Lyme disease could be driven by the positive effect of deer density on DON rather than by competent small mammals increasing NIP.

DIN varied across the season and I observed a peak in DIN for *B. afzelii* in September, which could affect human incidence. However, the risk would also depend on human activities. Indeed, even if the risk of contracting Lyme disease is found to be high in a particular spot (i.e. high DIN), it would translate to higher incidence of cases only if people are going to this area and encounter ticks and this is likely to be affected by weather, season for outdoor activities such as hill walking and mushroom picking as well as school holidays (Cairns *et al.*, 2019; Randolph *et al.*, 2008; Šumilo *et al.*, 2008; Trájer *et al.*, 2014).

When I focussed the analysis on the effect of deer on *B. burgdorferi* s.l. (i.e. all the genospecies combined), which represents the risk for humans, I found no association between DIN and the density of roe and red deer the previous year. These results imply that the role of deer in driving tick populations up and in lowering pathogen prevalence was at an equilibrium for *B. burgdorferi* s.l. This goes against my predictions as I would expect to see a similar pattern to the one I observed for *B. afzelii*. This analysis included genospecies transmitted by birds (*B. garinii* and *B. valaisiana*) so this disparity could be due to differences in competent reservoir hosts ecology and life history. One study found a positive correlation between DIN of *B. garinii* and roe deer density (Takumi *et al.*, 2019) and, to further test this, it would be interesting to survey the bird community as well. Due to the small infection rate of genospecies other than *B. afzelii* observed (*B. afzelii*: n=177 samples, *B. garinii*: n=48, *B. valaisiana*: n=26, *B. burgdorferi* s.s.: n=26), I could not run separate analyses to assess the impacts of deer and small mammals on DIN for these other genospecies.

This study is, to my knowledge, the first empirical survey that has demonstrated the complex and often opposing effects of the interaction between competent reservoir hosts (small mammals) and non-competent host (deer) over a wide range of deer densities to explain Lyme disease hazard. Additionally, the differences in

the effects of deer on DIN between *B. afzelii* and *B. burgdorferi* s.l. raise questions on whether the effects of deer on DIN might vary depending on the genospecies considered. While I could not explore things further, this should be addressed in future studies. While these results show how host community composition can affect Lyme disease hazard, a further question that is rarely considered is what affects host species composition and relative abundance, as this will have consequences for disease hazard.

With respect to small mammal abundance, several studies have shown that deer can strongly affect bird (DeCalesta, 1994; McShea and Rappole, 2000; Allombert *et al.*, 2005) and small mammal (Flowerdew & Ellwood, 2001; Smit *et al.*, 2011; van Wieren & Bakker, 2008) communities, either by direct disturbance and competition or through their impact on vegetation structure as sparser and shorter vegetation might increase predation risk on small mammals (van Wieren & Bakker, 2008). Similarly, there was a negative correlation between red deer and small mammal abundance (Figure 2.12) (Flowerdew & Ellwood, 2001; Smit *et al.*, 2011; van Wieren & Bakker, 2008) while roe deer density did not seem to affect small mammals. To my knowledge, this is the first empirical study showing that the effect of large ungulates on small mammal communities depends on deer species. Red deer are a gregarious species living in herds (Staines, 1976) and might have stronger impacts on vegetation and small mammal communities compared to roe deer, which are usually solitary or live in small family groups (Kjellander *et al.*, 2004; Vincent *et al.*, 1995). This implies that high densities of red deer could reduce NIP, not only by diverting ticks from feeding on competent reservoir hosts but also by reducing the number of competent reservoirs in the environment.

There was a negative correlation between roe and red deer (Figure 2.14) (Latham *et al.*, 1996; Latham & Staines, 1997), which could suggest interspecific competition. While this could be a result of differences in their ecology (Kamler *et al.*, 2008; Kleveland, 2007; Latham *et al.*, 1996; Mysterud, 1999; Reinecke *et al.*, 2014; Tufto *et al.*, 1996; Vincent *et al.*, 1995; Welch *et al.*, 1990), it could also be due to differences in deer management between roe and red deer. I also expected to see a landscape of fear modulated by human disturbance on the density of both red and roe deer. I found that roe deer density was negatively affected by the density of tracks in the study sites while it did not seem to affect

red deer (Bonnot *et al.*, 2013; Hewison *et al.*, 2001). This might be due to the difference in the ecology of these species but another study found that red deer avoided mountain biking trails (Scholten *et al.*, 2018), so I would expect them to be equally affected by human disturbance (Coppes *et al.*, 2017; Scholten *et al.*, 2018). I also expected to observe an effect of management type (i.e. forestry commission managed sites, hunting estates, private property or nature reserve) and, surprisingly, I did not see any differences in deer density but once again, it is likely to be due to the method used to estimate deer density. If anthropogenic pressure creates a landscape of fear, I would expect to see fewer deer when human activity is high and that would translate to fewer ticks. However, more of the remaining ticks might be infected as there should be more competent small mammals. What the impacts would be on Lyme disease hazard are unknown and could be addressed in future studies.

2.6. Study limitations

While this study was conducted across 28 sites and over a wide range of deer densities, providing a large sample size to study the effects of deer and small mammals on Lyme disease hazard, several caveats have been identified.

The low prevalence across the study area (mean NIP of 2%) did not provide the models with much variation and I could not investigate the effects of deer and small mammals on DIN for genospecies other than *B. afzelii* due to the low number of positive samples.

One of my main assumptions was that higher NIP and DIN would be seen in sites with high small mammal abundance. While my results suggest that it is not possible to find sites with high densities of both deer and small mammals, due to the negative effects of deer on small mammal communities, I should have observed higher NIP and DIN at sites with medium compared to low deer densities. I only trapped each site for two consecutive nights and excluded shrews while, ideally, I would have left traps inactivated for a few days before trapping and would have trapped for a longer period to increase small mammal sample sizes. If I had a larger sample size, I also would have been able to separate bank voles and wood mice in my analyses.

Because time and resources were limited, I could not add another year of sampling, which would have improved my sample size. Furthermore, I only looked over a short term window that does not tell me what had happened before I started or what would happened if there was a change in deer management, which is why more long term studies are needed. I am also aware that other competent reservoir hosts (e.g. birds, shrews and squirrels) were not included and that sheep and cattle, which were excluded to simplify this very complex system, are important in the epidemiological cycle and should be considered (Millins *et al.*, 2015; Ogden *et al.*, 1997; Salkeld *et al.*, 2008).

Lastly, I believe that the method I used to estimate deer density does not necessarily reflect how deer use their habitats. This issue has been highlighted by Hosmeester *et al.*, (2017) who pointed out that density estimation depends on scale as deer do not use their habitat homogeneously. Being able to combine pellet count with other methods such as tracking GPS collared deer would have allowed me to know more accurately how they are using their environment. However, this was not possible to do here due to logistic reasons. Another way to account for spatial heterogeneity using the data I collected would have been to include an interaction term between deer density and habitat type. While it was possible to implement this in the analysis focussing on nymph abundance, I was unable to add this interaction terms in the models focussing on NIP for *B. afzelii* and DIN as they would have been overparametrized. Even though deer density seemed to remain stable over the two years of data collection across sites, it would have been useful to have an estimate of deer density two years prior to tick collection as this would have reflected the time lag between adult female ticks feeding and the current cohort of nymphs.

2.7. Conclusion

To conclude, results from this study highlight the complexity of the Lyme disease epidemiological cycle. These results suggest that roe and red deer could play different roles and roe deer might be stronger dilution hosts. When considering *B. afzelii*, the role of deer as reproduction hosts appeared to be stronger than their role in mitigating disease prevalence through a dilution effect, as I observed an increase in DIN along increasing densities of both red and roe deer. However, when

considering *B. burgdorferi* s.l., which represents Lyme disease hazard for humans, there was no association between DIN and deer density and this should be addressed further to understand the impacts of host abundance on DIN for *B. garinii, B. valaisiana* and *B. burgdorferi* s.s. While this study shows how host community composition and the interaction between competent and non-competent hosts determine disease hazard, it also suggests that interspecific competition between host species and anthropogenic pressure could affect host abundance and host community structure and thus, Lyme disease hazard.

3. Experimental evidence for cascading effects of deer on Lyme disease pathogen, ticks and their rodent reservoirs

3.1. Abstract

Vector-borne pathogen transmission cycles rely on multiple hosts: competent reservoir hosts which maintain the pathogen and reproduction hosts which support vector populations. This is the case for the tick transmitted pathogen *Borrelia burgdorferi* sensu lato which causes Lyme disease. In this system, deer species act as tick reproduction hosts, thus driving vector populations and could increase Lyme disease hazard, defined as the density of infected nymphs (DIN). On the other hand, deer are also non-competent hosts and could reduce nymphal infection prevalence (NIP) for *B. burgdorferi* s.l. and thus, DIN, through a dilution effect. Lastly, high browsing pressure by deer could reduce the abundance of small mammals, which are competent reservoir hosts for *B. burgdorferi* s.l. and thus, lower DIN. In this study, I investigated the effect of high deer density on the ecological components contributing to Lyme disease hazard using a fencing experiment. It consisted of replicated areas in which red deer were absent or present at high density. Over four years, I surveyed questing ticks and assayed them for *B. burgdorferi* s.l. infection. Plots with high deer density were associated with an 18-fold increase in questing nymph density and a 2-fold decline in NIP. Overall, the positive effect of deer on tick abundance compensated for the lower NIP observed, as DIN was five times higher with deer than without. High deer density plots had 13 times fewer small mammals, likely linked to the lower and sparser ground vegetation recorded and this decrease in small mammal abundance could explain the lower NIP observed. Furthermore, a dilution effect might have also contributed to the lower NIP in high deer density plots as questing nymphs would have higher chances of feeding onto non-competent deer. These results suggest that the role of deer in driving tick populations can outweigh their role in lowering *B. burgdorferi* s.l. prevalence through a dilution effect and through their effects on small mammal abundance. This study shows how high deer density can impact tick-borne disease hazard through complex ecological cascades.

3.2. Introduction

Trophic cascades occur when a change in population or activity of a keystone species precipitates a cascade of alterations through trophic levels in an ecosystem (Paine, 1980). One classic example is the trophic cascade observed after the reintroduction of wolves (*Canis lupus*) in Yellowstone National park, US. The return of this apex predator led to a decline in elk (*Cervus elaphus*) populations, which reduced browsing pressure and increased aspen (*Populus tremuloides*) height and canopy cover, with a positive effect on beaver (*Castor canadensis*) populations (Beschta & Ripple, 2016; Ripple & Beschta, 2012).

While there are many examples of trophic cascades in ecology, their relevance for infectious disease ecology has received less attention. This could be particularly significant for vector-borne diseases, as trophic effects could arise from changes in both vector and reservoir host populations. A study conducted in 2017 showed that the introduction of the non-native Burmese python (*Python bivittatus*) in Florida, US resulted in a change in host community composition (Hoyer *et al.*, 2017). This caused the mosquito *Culex cedecei,* sole vector of the Everglade virus (causing non-fatal neurological diseases in humans) to shift its diet and resulted in an increase in blood meals taken from competent reservoirs hispid cotton rats (*Sigmodon hispidus*). This example illustrates the potential impacts of a trophic cascade on disease risk from the introduction of a predator. Downstream effects on vector-borne pathogen risk following changes in keystone species activity or populations are difficult to predict due to the complexity of trophic interactions and require long term studies to evaluate.

Lyme disease, the most prevalent vector-borne disease in humans in the northern hemisphere (Steere *et al.*, 2004; Walter *et al.*, 2017), is an emerging disease in Europe and North America caused by the *Borrelia burgdorferi* sensu lato complex of bacteria (hereafter referred to as *B. burgdorferi* s.l.) and transmitted by Ixodid ticks. In Europe, the primary vector is *Ixodes ricinus* (Stanek *et al.*, 2012), a generalist three-host tick that feed on a wide range of vertebrate species (Hofmeester *et al.*, 2016). There are several genospecies of the bacteria within the *B. burgdorferi* s.l. complex, each with different reservoir host associations: in Northern Europe, rodents are competent reservoir hosts for *B. afzelii* (Hanincová

et al., 2003a; Kybicová *et al.*, 2008) while some bird species are competent reservoir hosts for *B. valaisiana* and *B. garinii* (Hanincová, Taragelova, *et al.*, 2003b; Heylen *et al.*, 2014). *B. burgdorferi* sensu stricto, the fourth genospecies present, can be maintained by any competent reservoir host. Deer species, on the other hand, are non-competent hosts for *B. burgdorferi* s.l. (Brisson *et al.*, 2008; Cagnacci *et al.*, 2012; Pacilly *et al.*, 2014). However, they are important hosts for providing blood meals to adult female ticks prior to egg laying and immature tick life-stages (Hofmeester *et al.*, 2016; Humair *et al.*, 2007; Pichon *et al.*, 2005) and several studies have shown a positive association between deer density and the abundance of questing nymphs (Gilbert *et al.*, 2012; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014).

High deer densities can also affect vegetation and small mammal communities. Indeed, several studies have shown how browsing pressure by red deer (*Cervus elaphus)* and roe deer (*Capreolus capreolus*) can lead to sparser vegetation cover (Buesching *et al.*, 2011), changes in plant species richness in woodlands (Pellerin *et al.*, 2010) and changes in the abundance and community composition of birds (DeCalesta, 1994; McShea and Rappole, 2000; Allombert *et al.*, 2005) and small mammals (Dirzo *et al.*, 2014; Flowerdew & Ellwood, 2001; Keesing & Young, 2014; Smit *et al.*, 2011; van Wieren & Bakker, 2008) via direct competition and habitat modification.

Deer could therefore affect nymphal infection prevalence (NIP) with *B. burgdorferi* s.l. in different ways; firstly, high densities of deer could result in a rise in larva density, as more adult female ticks would have fed prior to laying eggs, which could increase encounter rates between larval ticks and competent reservoir hosts and potentially lead to higher NIP. On the other hand, deer could divert ticks from feeding on competent reservoir hosts and lower NIP, a concept called the dilution effect (Keesing *et al.*, 2010; LoGiudice *et al.*, 2003; Norman *et al.*, 1999; Ostfeld & Keesing, 2000b, 2000a; Schmidt & Ostfeld, 2001). Lastly, high deer density could also reduce NIP by reducing the abundance of small mammals in the environment through ecological cascades.

Even though the prevalence *B. burgdorferi* s.l. in the tick population is a key component for assessing the circulation of the pathogen within the environment, it cannot be directly related to Lyme disease hazard in said ecosystem (Logiudice *et al.*, 2008). Indeed, the likelihood of someone contracting Lyme disease will depend not only on the proportion of ticks being infected with *B. burgdorferi* s.l. but also on the probability of being bitten by a tick, which is reflected by the density of ticks (DON) in the environment. Thus, Lyme disease hazard is characterized by the density of infected nymphs (DIN) in the environment (Allan *et al.*, 2003; Kilpatrick *et al.*, 2017; Wood *et al.*, 2014). Deer could affect DIN by (1) driving tick populations, (2) lowering pathogen prevalence through a dilution effect and (3) through their effects on vegetation and small mamal communities, thus affecting competent reservoir host populations. There has been limited work investigating these effects simultaneously and testing the role of deer in potentially mitigating Lyme disease hazard. Therefore, this study investigated the effects of high deer density on *B. burgdorferi* s.l. transmission risk through ecological cascades by testing three hypotheses (Figure 3.1).

Hypothesis 1 – tick amplification hypothesis:

I expected to observe a positive correlation between deer density and questing nymph abundance due to deer acting as reproduction hosts (Gilbert *et al.*, 2012; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014) and feeding both adult female ticks and immature tick life-stages (Hofmeester *et al.*, 2016; Humair *et al.*, 2007; Pichon *et al.*, 2005). This increase in host seeking ticks should lead to higher tick burden on small mammals and thus, an increase in transmission potential. If this hypothesis is confirmed, I would further expect to observe a higher risk for Lyme disease transmission, due to a combined increase in NIP and DON in the environment (Figure 3.1).

Hypothesis 2 – dilution hypothesis:

I predicted that high deer density should divert questing ticks from feeding on competent reservoir hosts and thus, fewer should become infected. Thus, I predicted that I should observe a lower prevalence of *B. burgdorferi* s.l. when deer density is high (Figure 3.1).

Hypothesis 3 – habitat disturbance hypothesis:

I expected to observe an effect of browsing pressure by red deer on vegetation and I predicted shorter and sparser vegetation when deer density is high. This should result in a decline in small mammal abundance and thus, in a decrease in NIP as fewer questing ticks should feed on competent small mammals. This indirect impact of deer on small mammal community might also exacerbate the potential dilution effect by reducing the relative number of competent reservoir hosts available to feed questing ticks. If either of these last two hypotheses are confirmed, I would predict a low DIN when deer density is high (Figure 3.1). In order to test these hypotheses and tease apart the possible mechanisms involved for the effects of deer on the Lyme disease transmission cycle through ecological cascades, I used an experimental design of replicated fenced exclosures.

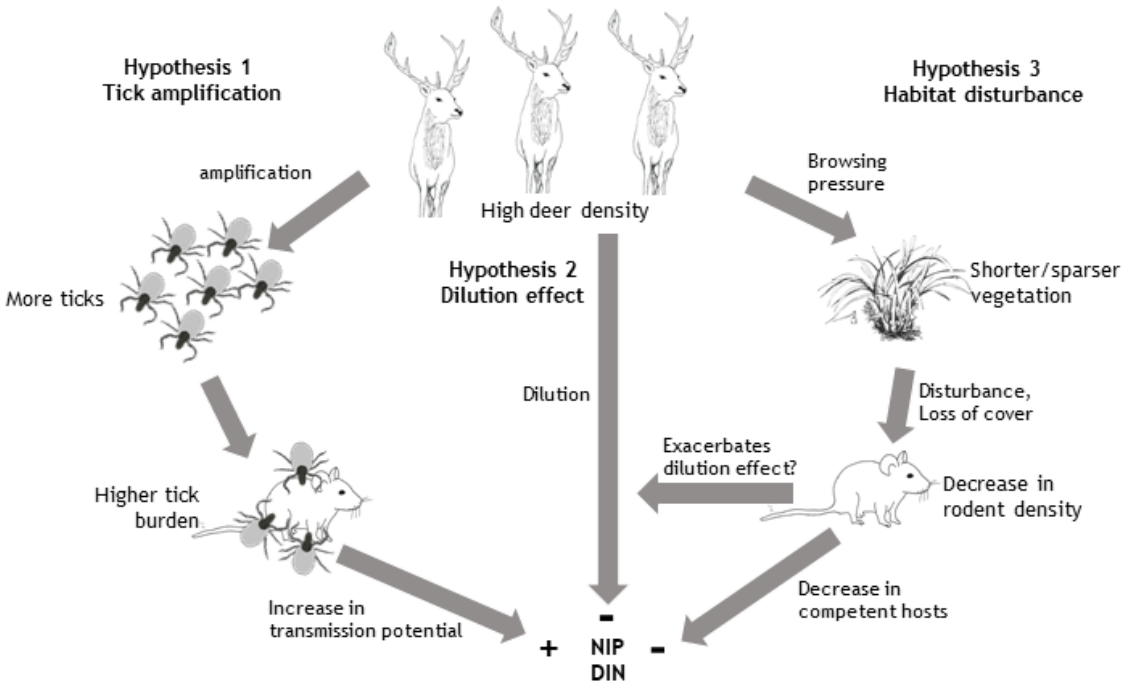


Figure 3.1: Conceptual figure to illustrate three hypothesised pathways through which high deer density might affect questing tick abundance (DON), nymphal infection prevalence (NIP) with *B. burgdorferi* s.l. and the density of infected ticks (DIN).

3.3. Materials and methods

3.3.1. Experimental design

The study took place at Glensaugh research farm in Aberdeenshire, North East Scotland (56.914°N, 5.532°W) in a 15 Ha enclosure of upland moorland where five red deer stags were kept (density equivalent to 32.47 deer/km^{-2}). Within this moorland enclosure, four replicates of fenced-unfenced pairs of plots of 0.25 Ha (124m x 20m) were set up in 2004/2005, initially to study the impact of deer browsing on woodland restoration. The four fenced plots (hereafter referred to as deer exclusion plots) excluded deer while the four unfenced plots (hereafter referred to as high deer density plots) were accessible to the deer and subjected to high browsing pressure (Figure 3.2 & Figure 3.3). For the purposes of the woodland restoration study, each plot was divided into five subareas of approximately 15m x 15m to create five habitats: high density birch, low density birch, a single birch in the centre of the plot, high density pine, and a control that was not planted and consisted of *Calluna vulgaris*-dominated heather moorland (Figure 3.2).Tree saplings were planted in 2004/2005 and were 9 years old by the time the current study started in 2013.

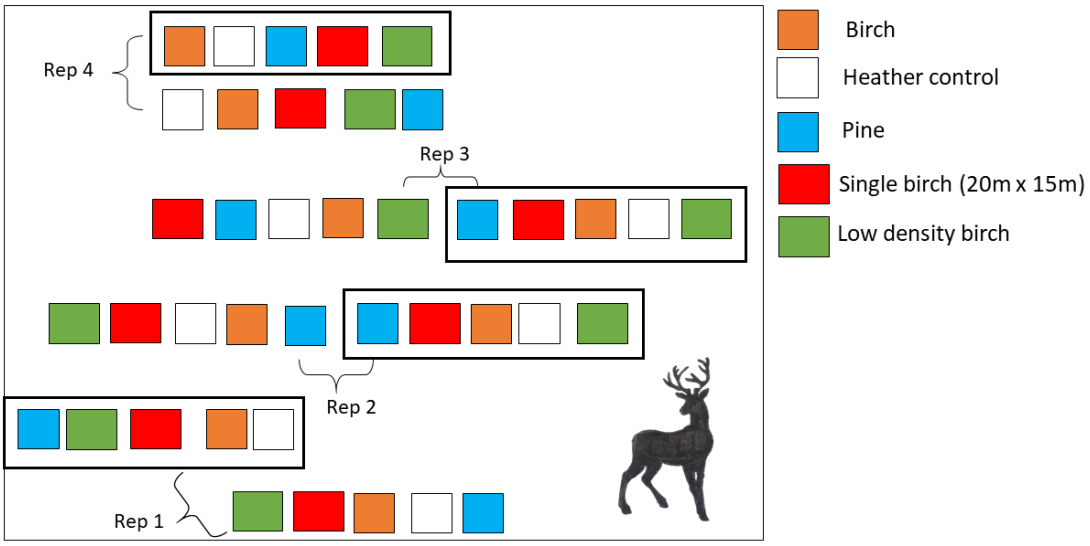


Figure 3.2: Conceptual figure to illustrate the experimental design. This experimental set up was conducted within a 15 Ha moorland enclosure consisting of 4 pairs of fenced/unfenced plots with 5 habitats created in each parcel (orange: high density birch trees, blue: high density pine trees, red: single birch tree, green: low density birch tree, white: heather control). The figure is not to scale.

Figure 3.3: Photograph showing one of the fenced enclosures (left) and one replicate of fenced and unfenced plot (right).

3.3.2. Questing *I. ricinus* nymph surveys

Questing nymphs were surveyed in 2013, 2014, 2018 and 2019 using a standard dragging method (Falco & Fish, 1992) which consists of dragging a 1m x 1m square of fleece blanket material over the ground vegetation for 10m linear transects. All nymph and adult ticks on the blanket were removed, counted and stored in Eppendorf tubes at -20°C until further analysis. In 2013 and 2014, 10 transects were surveyed in each of the heather, high density birch and pine habitats for all plots in May, July and September (n=720 transects per year) while six transects were surveyed in each of the same three habitats in 2018 and 2019 (n=432 transects per year). The sparse birch and single birch habitats were not surveyed for ticks in any year. Transects surveyed in deer exclusion plots were at least 2m away from the fence to avoid collecting ticks that might have crawled from outside the exclosure. Ticks were collected between 0900 hours and 1900 hours and air temperature, time, wind (categorised as weak: 0-12 kph, medium: 13-25 kph or strong: >25 kph), vegetation height, weather and relative humidity were recorded for each transect. Although I tried to conduct tick surveys on dry vegetation, it was not always possible so I recorded whether the ground was dry or not during collection.

3.3.3. Ground vegetation and tree survey

Ground vegetation for each transect was classified as one of two categories (grasses and herbaceous species or *Ericaceous* and V*accinium*). A sward stick was used to measure ground vegetation height and density at the beginning (1m),

middle (5m) and end (10m) of each transect. Coloured tape was placed every 5 cm on the sward stick and vegetation density was estimated by counting how many of these could not be seen when the stick was placed in the vegetation (Gilbert, 2010). Tree height was available for 2013 and 2014 (9/10 years after saplings were planted) for 205 birch trees and 160 pine trees (see Appendix B, S1 for more details). Vegetation height was available for all four years and vegetation density was available for 2013 and 2014.

3.3.4. Estimation of small mammal abundance

Small mammal trapping was conducted under the Home Office Regulations Project license 70/8543 and personal license I2B15B1E9. Small mammal abundance was estimated in July 2017 and July 2018 using a live-trapping method (Bouchard *et al.*, 2013; Quéré *et al.*, 2000). In 2017, four non-selective Sherman live traps (16 x 5 x 6.5 cm. HB Sherman Inc., Tallahassee, Flor.) equipped with shrew holes, so that shrews (*Sorex* spp.) could escape, were placed in each of the five habitats (heather control, high density pine, high density birch, sparse birch, single birch) within each plot with 10m spacing, resulting in a total of 20 traps per plot. Traps were baited with oats and bedding material was provided. Traps were set for three consecutive nights in two of the paired fenced/unfenced plots (replicates 1 and 2, see Figure 3.2) and for two consecutive nights in the other two paired fenced/unfenced plots (replicates 3 and 4, see Figure 3.2) for a total of 389 trap nights. In 2018, four traps were installed for four consecutive nights in the three habitats in which ticks were surveyed (heather control, high density birch and pine habitats) in each plot, resulting in 12 traps per plot and a total of 384 trap nights. Traps were activated after 1600 hours and checked every morning before 1000 hours. For all captures, species, sex, weight and age category (juvenile or adult) of each individual were recorded. All ticks attached to small mammals were counted and collected from around the head and ears and stored in 100% ethanol. A skin biopsy was taken from each individual upon first capture using an ear punch and stored in 100% ethanol (Sinsky & Piesman, 1989). All captured small mammals were released at the capture site.

3.3.5. DNA extraction and estimation of *B. burgdorferi* s.l. prevalence

DNA was extracted from questing nymphs by individually placing them in an Eppendorf tube with 0.7M NH_4OH, heating them at 100°C in for 15 min, centrifuging them and boiling them again with the lid open until 50µL of solution was left in tubes (Gern *et al.*, 2010). One negative control was included for every 39 samples. DNA from small mammal skin biopsies was extracted using the Qiagen DNeasy Blood and Tissue kit following the manufacturer's instructions (Qiagen, Hilden, Germany).

B. burgdorferi s.l. was detected using either a nested PCR or qPCR. Ticks collected in 2013 and 2014 were tested using a nested PCR targeting the 5S-23S intergenic spacer region (Rijpkema *et al.*, 1995). The multimix contained 10x PCR buffer, 2.5mM $MgCl_2$, 0.2mM deoxynucleotide, 0.625U of Taq DNA polymerase, 2.5pM of primers for each round (Table 3.1). Products obtained after the first round were diluted 1 in 10 and 1 µL was used for the second round. The program for the first PCR step consisted of 1min at 94°C followed by 29 rounds of temperature cycling (94°C for 30s, 52°C for 30s and 72°C for 1 min) while the program for the second round consisted of 1 min at 94°C (denaturation) followed by 39 rounds of temperature cycling (94°C for 30s, 55°C for 30s, and 72°C for 1 min). DNA from *B. lusitaniae* was used as a positive control as this genospecies is not found in Scotland. Samples were visualized on 2% agarose gel containing ethidium bromide in Tris-borate-EDTA buffer.

Samples collected in 2018 and 2019 were tested using a qPCR (Heylen *et al.*, 2013) which detects fragments of the OspA gene (Table 3.1) and optimized using the IQ™ Supermix (Bio-Rad Laboratories, Hercules, USA) in a Stratagene Mx3005P thermal cycler (Agilent, Santa Clara, US). Each reaction contained IQ™ Supermix, two primers at 200nM (Table 3.1), the probe at 100nM (Table 3.1) and 3µL of DNA. One positive and one negative control were added for every plate. I confirmed thee correspondence between the two PCR protocols by using 61 known positive samples of *B. afzelii*, *B. valaisiana*, *B. garinii* and *B. burgdorferi s.s* and 344 known negative samples. In all cases, results from the qPCR matched those of the nested PCR.

Table 3.1: Names and sequences of primers and probe used in the nested PCR and qPCR protocols

Protocol		Designation	Sequence
Nested PCR[a]	1st round	Primer 23SN1	5'-ACCATAGACTCTTATTACTTTGAC
		Primer 23SC1	5'-TAAGCTGACTAATACTAATTACCC
	2nd round	Primer 23SN2	5'ACCATAGACTCTTATTACTTTGACCA
		Primer 5SCB	5'-biotin-GAGAGTAGGTTATTGCCAGGG
qPCR[b]		Primer B-OspA_modF	AATATTTATTGGGAATAGGTCTAA
		Primer B-OspA_borAS	CTTTGTCTTTTTCTTTRCTTACAAG
		B-OspA_mod-probe	FAM-AAGCAAAATGTTAGCAGCCTTGA-BHQ-1™

[a] Method from Rijpkema *et al.*, 1995. [b] Method from Heylen *et al.*, 2013.

In order to identify the genospecies, positive *B. burgdorferi* s.l. samples from the qPCR protocol were subjected to the nested PCR protocol. Every positive sample was separated by a negative control to avoid contamination. All PCR products from positive samples were sent to Edinburgh Genomics for Sanger sequencing to identify the genospecies of *B. burgdorferi* s.l. present.

3.3.6. Statistical analyses

All statistical analyses were performed in R version 3.5.1 (R core team, 2013) using the glmmTMB (Brooks *et al.*, 2017; Magnusson *et al.*, 2019), lme4 (Bates, 2010) and MuMIn (Barton, 2019) packages.

For all the analyses using generalized linear mixed effect models (GLMMs) and generalized linear models (GLMs), the same method was followed. After identifying an appropriate distribution for each response variable, I assessed for potential collinearity between covariates by calculating variance inflation factors (VIFs) and variables with VIFs above 4 were discarded from the models (Zuur *et al.*, 2009). I used an F-test to assess whether continuous variables should be left in their linear forms or if polynomial relationships with the response variable could be identified. Each continuous variable was tested in both forms against the response variable and model fit was assessed. I tested for potential overdispersion of the data by looking at the ratio between the residual deviance and the residual degrees of freedom (Harrison, 2014). If the ratio was above 1, it indicated overdispersion and an observation level random effect was included for models using Poisson (Harrison, 2014) and Binomial (Harrison, 2015) distributions.

If the response variable was a count, I used a GLMM with a Poisson distribution and I also fitted zero-inflation and hurdle models with a Poisson distribution, as the number of zeros might have been underestimated, and chose the most adequate model based on AICc .

Model simplification was done by either using a backward stepwise regression until all variables left significantly improved model fit or using the dredge function from the MuMIn package (Barton, 2019) to test all variable combinations possible and I chose the model with the lowest AICc (Brewer *et al.*, 2016; Burnham & Anderson, 2004; Johnson & Omland, 2004; Zuur *et al.*, 2009). I used the backward stepwise regression method when the model had too many variables for the dredge function to run. The conditional R^2 was calculated using the MuMIn package when possible (it is not yet implemented for zero inflation and hurdle GLMMs) (Johnson, 2014; Nakagawa & Schielzeth, 2013). Finally, I used post-hoc comparisons using the Tukey HSD test with the Hold-Bonferroni correction to assess pairwise association across levels of categorical variables.

3.3.6.1. The effects of high deer density on questing nymph abundance (DON)

To assess how high deer density affected questing nymph abundance (DON), I used a hurdle GLMM with a Poisson distribution. The response variable was the number of questing nymphs collected per transect and the full model included plot treatment (deer exclusion or high deer density), month (May, July or September), year (2013, 2014, 2018 or 2019), habitat (high density pine, high density birch or heather control), vegetation height, relative humidity, temperature, whether the ground was dry during collection and the interactions between plot treatment and month and between plot treatment and habitat. Indeed, I expected that the effect of deer on tick density might differ between months as more ticks would be picked up by deer along the season in high deer density plots. Regarding the second interaction, the effect of habitat on the response variable might depend on plot treatment (e.g. higher trees in deer exclusion plots). Wind was not included as it was correlated with year (VIF=5). Randoms effects of a combined plot and habitat level (e.g. high density birch in fenced replicate 1) and an observation level random effect were included. I scaled continuous terms to help with model convergence and I used a backward stepwise regression for model simplification.

3.3.6.2. The effects of high deer density on ground vegetation and tree height

In order to assess how browsing pressure by red deer affected ground vegetation structure and trees, I compared the mean ground vegetation height, vegetation density and tree height between deer exclusion and high deer density plots. Because the two independent groups were not normally distributed, I used a non-parametric Mann Whitney for ground vegetation density. Vegetation height and tree height in deer exclusion and high deer density plots were normally distributed so I used a parametric z-test to compare their means.

3.3.6.3. The effects of high deer density on small mammals

To assess how grazing pressure by red deer affected small mammal abundance, I used a binomial GLM with a logit-link. The response variable was the proportion of active traps per habitat that had captured a small mammal and the full model included plot treatment, habitat and year as fixed covariates. I used the dredge function for model selection.

3.3.6.4. The effects of high deer density on tick burden on bank voles

I used data from a reference study, for which small mammal trapping and questing tick surveys were conducted in woodlands in North East Scotland to compare larval burden between bank voles caught in natural forests (reference study, data from Chapter 2) and those trapped in deer exclusion plots. I only used trapping data for bank voles as it was the only species captured in this study (see section 3.4.3). Because groups were not normally distributed, I used Mann-Whitney tests to compare the average larval burden on male and female bank voles between these two studies.

3.3.6.5. The effects of high deer density on *B. burgdorferi* s.l. prevalence in questing nymphs (NIP)

A major aim of this study was to test the hypothesis that deer may act as potential dilution hosts for *B. burgdorferi* s.l., which represents Lyme disease hazard for humans, so I pooled observations for the three genospecies found (*B. afzelii, B. garinii* and *B. valaisiana*). The response variable was the proportion of questing nymphs infected and I used a binomial GLMM with a logit-link (3 prevalence

estimates per habitat and per year). I found a strong correlation between plot treatment and small mammal abundance (VF=61) and ground vegetation height (VIF=52) so I did not include these variables in the model. I also found a correlation between year and the PCR method used (VIF=17) so I only kept year in the model. The full model included plot treatment, month, habitat and year. An observation level random effect was included and I used the dredge function for model selection.

3.3.6.6. The effects of high deer density on the density of questing nymphs infected with *B. burgdorferi* s.l. (DIN)

The response variable was the density of infected nymphs (DIN) and, as this variable was averaged at the plot level (3 estimates per habitat and per year), I used an offset for the area to transform the values to integers (i.e. if 6.5 nymphs were infected/1000m^{-2}, it was transformed as 7 nymphs infected/1076m^{-2}, see Zuur *et al.*, 2009). I used a hurdle GLMM with a Poisson distribution and the full model included plot treatment, month, year, habitat, temperature and whether the ground was dry during tick survey as fixed variables. An observation level random effect was included. Vegetation height (VIF=52) and small mammal abundance (VIF=61) were correlated with plot treatment and the PCR method used with year (VIF=17) and were not included in the model. I used the dredge function for model selection.

3.4. Results

3.4.1. The effects of high deer density on questing nymph abundance (DON)

After four years of data collection, 12,310 questing nymphs were counted (n=2,135 transect surveyed). A random subset of 1,000 nymphs collected in this experimental design was examined under the microscope for species identification using specific keys (Marquez *et al.*, 1992) and all were found to be *Ixodes ricinus*. Thus, I assumed that all questing ticks collected in this study were *I. ricinus*.

The final model included plot treatment, month, year, habitat, relative humidity, temperature and whether the ground was dry for the conditional part of the model

and plot treatment, month, habitat, year, temperature and whether the ground was dry in the zero-inflation part of the model. The full summary table is available in supplementary materials (Appendix B, S2) and Table 3.2 shows the contribution of each variable in the model.

On average, 18 times more nymphs were collected per 10m in high deer density plots (predicted DON= 10.9, 95%CI: 0.1-36) compared to deer exclusion plots (predicted DON= 0.6, 95%CI: 0-3.4) (Table 3.2 & Figure 3.4). DON was also higher in July (predicted DON=7.4, 95%CI: 0.1-25.1) compared to May (predicted DON=4.6, 95%CI: 0-16.6) and September (predicted DON=4.9, 95%CI: 0-17.0) and more ticks were sampled in high density birch (predicted DON=7.3, 95%CI: 0.1-25.3) and pine (predicted DON=6.5, 95%CI: 0.1-22.2) habitats compared to heather (predicted DON=3.4, 95%CI: 0-12.1) (Appendix B, S2).

Table 3.2: Summary table from the conditional section of selected model showing which variables influenced DON. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

Variables	ΔAICc	Δlog-Lik	Δdf
Plot treatment	74.3	38.1	1
Month	16.0	10.0	2
Year	142.7	74.4	3
Habitat	19.6	11.8	2
Relative humidity	27.2	14.6	1
Ground wetness	5.5	3.8	1
Temperature	4.7	3.3	1

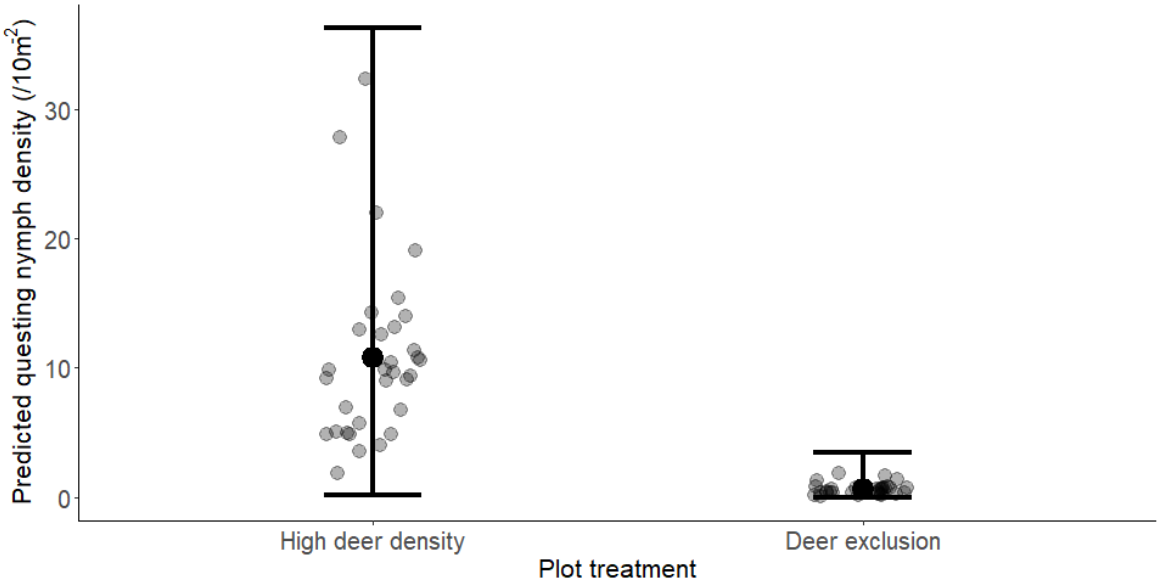


Figure 3.4: Predicted density of questing nymphs per 10m ±95% CI in high deer density (32.47 deer/km^{-2}) and deer exclusion plots.

3.4.2. The effects of high deer density on ground vegetation and tree height

Vegetation was significantly denser in deer exclusion plots (median=3.0) compared to high deer density plots (median=2.0) (Mann-Whitney, U=574570, $n_1=n_2=863$, $p<0.001$ two-tailed, Figure 3.5-B). The same difference was observed for vegetation height, which was, on average, 14.4 cm shorter in high deer density plots compared to deer exclusion plots ($z=31.32$, $p<0.001$, Figure 3.5-A). Trees in deer exclusion plots were, on average, 17 cm taller than those in high deer density plots ($z=2.79$, $p=0.005$,Figure 3.5-C).

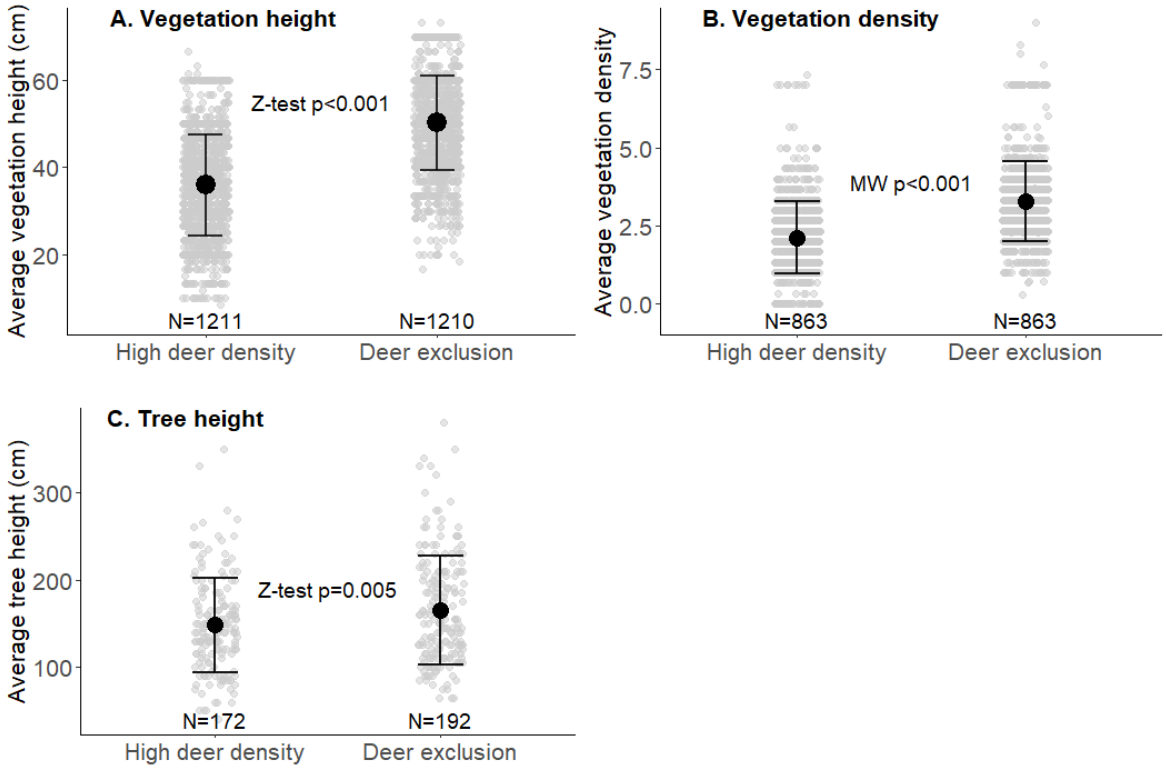


Figure 3.5: Average vegetation height (A), vegetation density (B) and tree height (C) in high deer density and deer exclusion plots. Error bars represent standard deviations.

3.4.3. The effects of high deer density on small mammals

In both 2017 and 2018, 12 individual bank voles (2017: 8 males and 4 females, 2018: 4 males and 8 females) were caught in deer exclusion plots while only one was captured in high deer density plots (2017: 1 male, 2018: 1 female). No other small mammal species were trapped. During the 2017 trapping season, six traps did not activate, resulting in a total of 389 trap nights in 2017 and 384 in 2018.

The model selection suggested that bank vole abundance was associated with plot treatment only and, on average, 13 times more bank voles were predicted to be caught per 100 trap nights in deer exclusion plots (predicted bank vole abundance=6.6/100TN, 95%CI: 4.5-9.5) compared to high deer density plots (predicted bank vole abundance=0.5/100TN, 95%CI: 0.1-2.0) (Table 3.3 & Figure 3.6). Overall, the best model (i.e. plot treatment only) explained 36% of the variance in bank vole abundance.

Table 3.3: Summary table from the selected model showing variables influencing bank vole abundance (proportion of traps with captures). ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	-5.27	0.71	-		
Plot treatment: deer exclusion	2.62	0.74	49.7	25.8	1

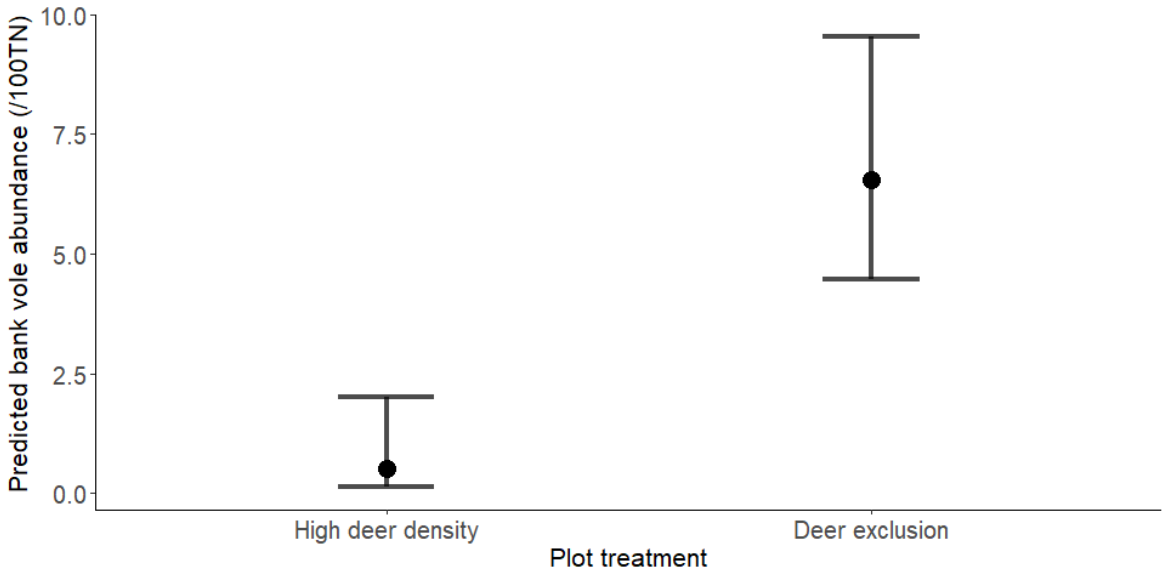


Figure 3.6: Predicted abundance of bank voles per 100 trap nights ±95% CI in high deer density (32.47 deer/km^{-2}) and deer exclusion plots.

3.4.4. The effects of high deer density on tick burden on bank voles

Over both years of small mammal trapping, only two bank voles were captured in high deer density plots so I could not meaningfully compare larval burdens on bank voles between deer exclusion and high deer density plots.

Male bank voles captured in deer exclusion plots harboured significantly more larvae (median=20.5) compared to those in natural woodlands (median=2), using data from a reference study (Mann-Whitney, U=28.5, n_1=12, n_2=21, $p<0.001$, Figure 3.7). Female bank voles in deer exclusion plots had higher larval burdens to those in the reference study (deer exclusion plots: median=5, reference study: median=1) but this differences was not significant (Mann-Whitney, U=28.5, n_1=12, n_2=22, p=0.12, Figure 3.7). Questing nymph density during small mammal trapping ranged between 0.05 to 3.23 per $10m^{-2}$ in the reference study and from 0.17 to 1.35 per $10m^{-2}$ in deer exclusion plots (Table 3.4).

Table 3.4: Summary of the deer density (deer/km^{-2}), density of questing nymphs/10m (DON), number of bank voles captured and larval burden on individual bank voles data from the reference study conducted in 12 woodland sites in 2017 and 2018 (data from Chapter 2) compared to deer exclusion plots and high deer density plots for reference. $\bar{x}$ is the mean and med the median.

Study	Deer density	DON	No. of bank voles	Larval burden on voles
Reference study	0-19.0 $\bar{x}$=12.3 med=15.1	0.05-3.2 $\bar{x}$=1.6 med=2	43 bank voles (22♀ and 21♂)	0-30 $\bar{x}$=4.2 med=1.0
Deer exclusion plots	0	0-12 $\bar{x}$=0.5 med=0	24 bank voles (12♀and 12♂)	0-40 $\bar{x}$=14.2 med=12
High deer density plots (for reference)	32.47	0-169 $\bar{x}$=11.0 med=7.0	2 bank voles (1♀ and 1♂)	10-25 $\bar{x}$=17.5 med=17.5

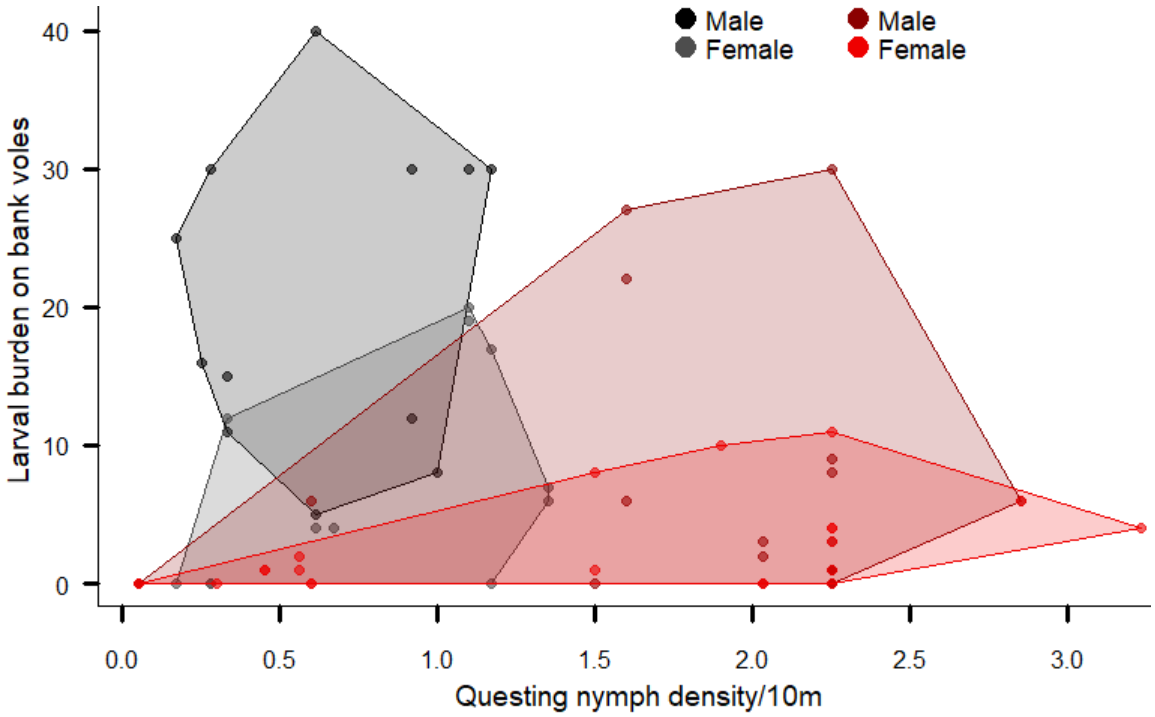


Figure 3.7: The relationship between larval burden on bank voles and questing nymph density from blanket drags for male (dark grey) and female (light grey) bank voles in deer exclusion plots and for male (dark red) and female (light red) bank voles in natural woodlands in Aberdeenshire. Larval burdens were expected to increase with tick abundance, measured here as nymph density. Relative to the low tick abundance in deer exclusion plots, bank voles captured in these plots had relatively high tick burdens, which was particularly evident for the males and may suggest movement outside the deer exclusion plots to high density deer plots with increased questing tick density.

Skin biopsies assays showed that 8.3% (n=2/24) of bank voles from deer exclusion plots were infected with *B. afzelii* compared to 100% (n=2/2) from high deer density plots.

3.4.5. The effects of high deer density on *B. burgdorferi* s.l. prevalence in questing nymphs (NIP)

Out of the 12,310 questing nymphs collected over four years, all ticks collected from deer exclusion plots (n=585) and 1,042 (out of 11,725) from high deer density plots were tested for *B. burgdorferi* s.l. I used a power analysis to decide the number of questing nymphs collected in high deer density plots needed to be tested, using prevalence data from 2013 and 2014 to calibrate the power analysis in terms of effect size. Overall, 2.7% of nymphs (n=16/585, 95%CI: 1.6-4.4) were infected with *B. burgdorferi* s.l. in deer exclusion plots while 0.9% (n=9/1,042, 95%CI: 0.4-1.6) were infected in high deer density plots (Appendix B, S4). The most prevalent genospecies was *B. afzelii* (92% of infected nymphs, n=23) followed by *B. valaisiana* (4%, n=1) and *B. garinii* (4 %, n=1).

The simplified model included plot treatment, month, habitat and year. NIP was not significantly different between deer exclusion (predicted NIP=2.2, 95%CI: 0.6-7.6) and high deer density plots (predicted NIP=1.0, 95%CI: 0.3-4.2) (Table 3.5 & Figure 3.8 & Appendix B, S5). NIP was higher in September (predicted NIP=3.1, 95%CI: 1.0-10.1) compared to May (predicted NIP=0.5, 95%CI: 0.1-2.8) but similar to July (predicted NIP=1.3, 95%CI: 0.3-5.0) while it did not differ between years and habitats (Appendix B, S5). Overall, the model explained 37% of the variance. The same results were obtained when looking at NIP for *B. afzelii* alone (Appendix B, S6).

Table 3.5: Summary table from the selected model showing which variables were associated NIP for *B. burgdorferi* s.l. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	-6.20	1.00			
Plot treatment: deer exclusion	0.77	0.52	0.4	1.2	1
Month			4.5	4.3	2
May	-1.07	0.83			
September	0.90	0.50			
Habitat			1.9	3.0	2
Heather	1.05	0.70			
Pine	1.45	0.62			
Year			2.6	4.3	3
2014	0.72	0.74			
2018	-1.03	0.95			
2019	1.02	0.70			

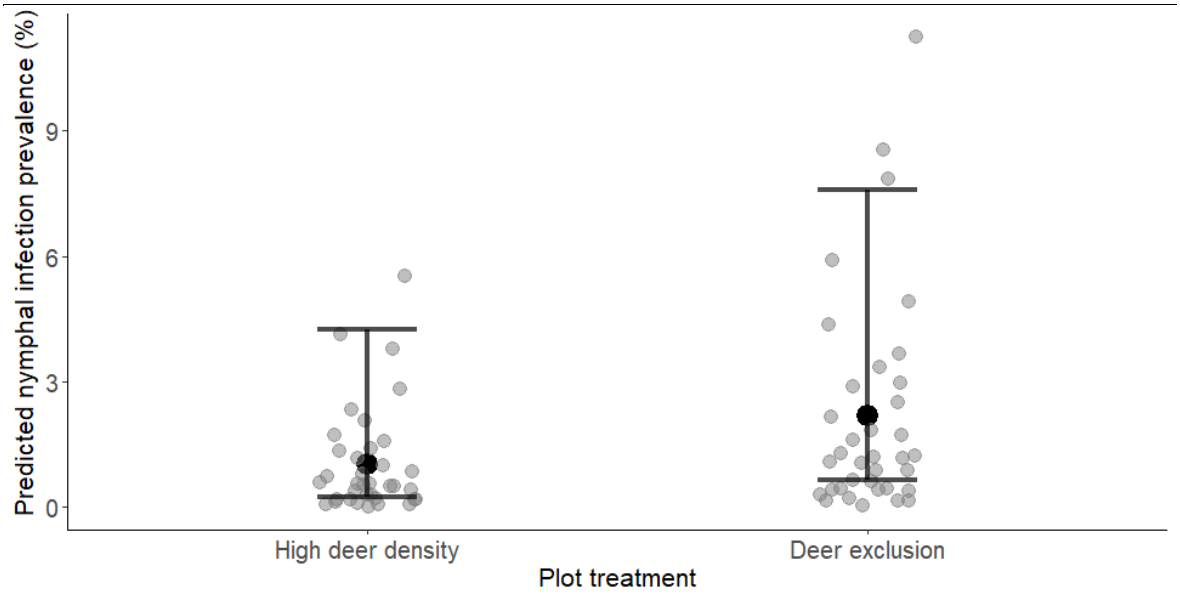


Figure 3.8: Predicted nymphal infection prevalence for *B. burgdorferi* s.l. (%) ±95% CI in high deer density (32.47 deer/km^{-2}) and deer exclusion plots.

3.4.6. The effects of high deer density on the density of questing nymphs infected with *B. burgdorferi* s.l. (DIN)

The simplified model included plot treatment, habitat and temperature in the conditional part of the model and whether the ground was dry during tick surveys in the zero-inflation part (Table 3.6 & Appendix B, S7). DIN was five times higher in high deer density plots (predicted DIN=5.1, 95%CI: 0-30.8) compared to deer exclusion plots (predicted DIN=1.0, 95%CI: 0-6.7) (Table 3.6 & Figure 3.9). DIN was also higher in pine (predicted DIN=4.9, 95%CI: 0-28.9) compared to heather (predicted DIN=2.3, 95%CI: 0-14.2) and birch (predicted DIN=2.1, 95%CI: 0-13.5) habitats but similar between years (Appendix B, S8).

Table 3.6: Summary table from the conditional section of selected model showing which variables were associated with DIN for *B. burgdorferi* s.l. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	-5.85	0.75			
Plot treatment: deer exclusion	-1.76	0.28	17.5	9.8	1
Habitat			2.9	3.4	2
Heather	0.05	0.42			
Pine	0.83	0.39			
Temperature	0.10	0.05	1.9	1.9	1

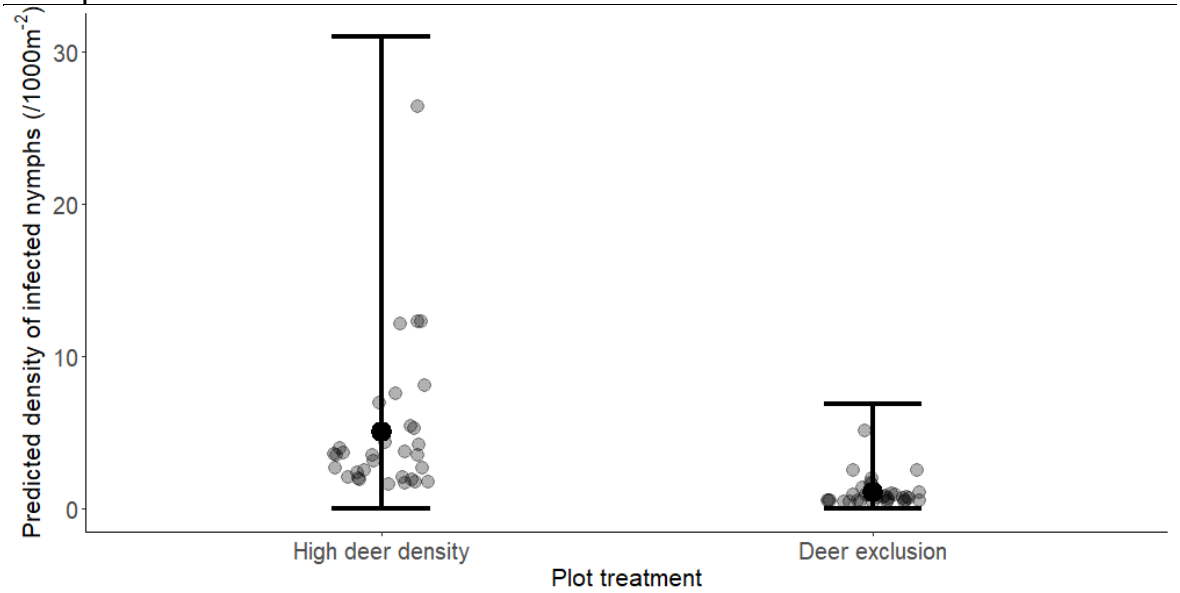


Figure 3.9: Predicted density of nymphs infected with *B. burgdorferi* s.l. per 1000m^{-2} ±95% CI in high deer density (32.47 deer/km^{-2}) and deer exclusion plots.

3.5. Discussion

This experimental design offered a chance to investigate multiple ways in which high deer density might impact vector and reservoir host populations and thus pathogen transmission and disease hazard. I found strong indications that such effects exist at multiple levels and are changing transmission dynamics and pathogen prevalence. Browsing pressure by red deer had a strong, negative, impact on vegetation structure and tree height, which led to a decrease in bank vole abundance and resulted in a reduction in the prevalence of *B. burgdorferi* s.l., albeit non-significant. On the other hand, more questing nymphs were collected in high deer density plots, demonstrating their role in driving tick populations up. The way in which these different effects interact can lead to unexpected outcomes with respect to disease hazard. Indeed, I expected high deer density to cause a reduction in Lyme disease hazard due their negative effect on competent reservoir hosts and through a dilution effect. However, DIN was five times higher in high deer density plots, suggesting that their role in driving tick populations outweighed their negative effect on competent reservoir hosts in this experimental study.

Regarding the first hypothesis (tick amplification), 18 times more nymphs were collected in high deer density plots compared to deer exclusion plots (Figure 3.4). However, this is not a direct relationship as there will be a lag between deer density and DON (questing nymphs from $year_t$ would have fed on deer as larvae at $year_{t-1}$ or come from adult females that fed at $year_{t-2}$ before laying their eggs) and other factors such as temperature (Bertrand & Wilson, 1996; Ruiz-Fons *et al.*, 2012), humidity (Knap *et al.*, 2009; Milne, 1950; Randolph & Storey, 1999), habitat (Dobson *et al.*, 2011b; Lindström & Jaenson, 2003) and ground vegetation (Milne, 1950; Pfäffle *et al.*, 2013) can affect tick survival and questing behaviour. Still, these results highlight the importance of deer in feeding adult female ticks (Gilbert *et al.*, 2012; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014), which can then reproduce and lay eggs but also their role in feeding larvae (Hofmeester *et al.*, 2016; Humair *et al.*, 2007; Pichon *et al.*, 2005), as almost no bank voles were captured in high deer density plots. I did not survey birds or shrews during this study and they are known hosts for larval ticks (Brisson *et al.*, 2008; Hofmeester *et al.*, 2016; Klaus *et al.*, 2016; Millins, 2016) but several studies have found a

negative effect of high deer density on both birds (Gill & Fuller, 2007; Holt *et al.*, 2011; McShea & Rappole, 2000) and shrews (Flowerdew & Ellwood, 2001; Herder *et al.*, 2016), so I assume that their populations were low in high deer density plots and that the majority of larvae probably fed on red deer.

This contrast in tick density should have been reflected on tick burden on bank voles and I expected to observe higher larval burdens on individuals caught in high deer density plots compared to deer exclusion ones. Unfortunately, I could not make this comparison as only two bank voles were caught in high deer density plots over two years of trapping. When I compared larval burden on bank voles caught in deer exclusion plots to those of bank voles captured in natural woodlands (reference study), I observed that males had higher burdens compared to their conspecifics in forests for similar densities of nymphs (Figure 3.7). I hypothesise that males probably that ventured outside of deer exclusion plots to forage might have larger home ranges compared to females (Rooney *et al.*, 1998), who had similar tick burden compared to those trapped in the reference study. If bank voles venturing outside of deer exclusion plots had higher individual tick burden, this would be in line with my prediction that high deer density increases contact rate between ticks and small mammals (hypothesis 1) and this should be investigated further.

Both bank voles caught in high deer density plots were infected with *B. afzelii* while only 2 out of 24 were infected in deer exclusion plots. Due to the low number of observations, I could not explore things further but several hypotheses could explain this difference; the first one could be that these two individuals were foraging outside of deer exclusion plots when captured. A second explanation could be that these two individuals permanently resided in high deer density plots and acquired the infection there, which would be in line with my predictions that high deer density increases transmission potential.

When I investigated the effects of high deer density on the prevalence of *B. burgdorferi* s.l. in questing nymphs, I expected to either see an increase in NIP (hypothesis 1) or a decrease due to a dilution effect of deer (hypothesis 2) (Huang *et al.*, 2018; Rosef *et al.*, 2009; Wood *et al.*, 2014) or through habitat modification (hypothesis 3). Although NIP for *B. burgdorferi* s.l. was low across all plots, it was

lower in high deer density plots (1% predicted in high deer density plots vs 2.2% predicted in deer exclusion plots, Figure 3.8). This difference was not statistically significant but it supports hypotheses 2 and 3 and brings evidence for the role that high deer density might play in mitigating pathogen prevalence.

Even though NIP was low, *B. burgdorferi* s.l. circulated in high deer density plots. Since the majority of nymphs that tested positive were infected with the rodent associated pathogen, *B. afzelii* (92%), I can assume that birds did not contribute much in pathogen transmission in this system. Based on the comparison of larval burden on bank voles from this experiment to those of bank voles captured in woodlands, I hypothesis that bank voles might be moving between deer exclusion and high deer density plots (Figure 3.7). Thus, bank voles residing in deer exclusion plots could be dropping infected larvae in high density plots while foraging for food or moving between fenced areas. These results are consistent with the first hypothesis, predicting an increase in contact rate between small mammals and ticks in high deer density plots and different mechanisms are likely to be involved.

In addition to their role in driving tick populations and lowering pathogen prevalence, high deer density resulted in sparser and shorter vegetation as well as shorter trees, highlighting the effects of deer grazing on vegetation structure (Figure 3.5) (Buesching *et al.*, 2011; Pellerin *et al.*, 2010; Smit *et al.*, 2011). In line with my predictions (hypothesis 3), fewer bank voles were captured in high deer density plots (Figure 3.6: abundance was 13 times lower) (Dirzo *et al.*, 2014; Flowerdew & Ellwood, 2001; Keesing & Young, 2014; Smit *et al.*, 2011; van Wieren & Bakker, 2008) and a likely mechanism for this effect could be that the denser and higher ground vegetation that I recorded in deer exclusion plots provided shelter and protection from predators (Eccard *et al.*, 2008; Korpimäki *et al.*, 1996; Pusenius & Ostfeld, 2002). In addition, deer trampling and direct competition for resources could have had a negative impact on bank vole abundance in high deer density plots. In the context of disease ecology, small mammals are competent reservoir hosts for many pathogens (Holmberg *et al.*, 2003; Mills *et al.*, 1999; Reed *et al.*, 2003; Riehm *et al.*, 2011) and an ecosystem supporting high density of deer could result in lower prevalence of pathogens, thus decreasing contact rate between infected small mammals and vectors or between infected small mammals and humans (Mills *et al.*, 1999; Olsson *et al.*, 2003).

Regarding the density of infected nymphs, which is the key outcome I was interested in, DIN was five times higher in high deer density plots (Figure 3.9), indicating that Lyme disease hazard was higher in high deer density plots compared to deer exclusion ones, even though NIP was lower in the former. These results are in line with those of Takumi *et al.* (2019) and Vourc'h *et al.*, (2016) who both found a positive correlation between DIN and roe deer density. This implies that, within this experimental study, the role of deer as reproduction hosts, driving tick populations up is more important in the context of Lyme disease hazard than their potential role as a dilution host.

High deer density resulted in higher DIN, providing evidence for the first hypothesis (increase in transmission potential through tick amplification). Several studies have shown that higher densities of competent reservoir hosts are associated with higher DIN (Ostfeld *et al.*, 2018b; Takumi *et al.*, 2019; van Duijvendijk, 2016). Thus, I should have observed a higher DIN in deer exclusion plots, where competent bank voles were more abundant but it was not the case in this study. Regardless of NIP, the positive association between competent reservoir hosts and DIN is likely to happen only if there are enough vectors in the environment to transmit pathogens (Logiudice *et al.*, 2008) and Lyme disease hazard would be the highest in an environment supporting both high number of competent reservoirs and enough reproduction hosts. Based on results from this study, it is unlikely to happen as high deer densities had strong, negative effects, on small mammal abundance (Flowerdew & Ellwood, 2001; Smit *et al.*, 2011; van Wieren & Bakker, 2008). However, this should be investigated at a larger scale as we are likely to see a combination of the different mechanisms proposed here.

3.6. Study limitations

While this study stresses the impacts of high browsing pressure by red deer on ground vegetation and tree height, the nature of the effect could depend on the species of grazer. For example, high densities of roe deer could have a different effect since both species have different feeding ecology, roe deer being more selective while red deer are non-selective grazers (Latham *et al.*, 1999; Storms *et al.*, 2008). In this experiment, deer were fenced within a 15 Ha enclosure thus, limiting their movements, and might not display the same feeding behaviour as

they would if they were free-living. Indeed, some studies have shown that red deer change their diet depending on the season and usually feed on grass during summer months while they favour heather in winter (Latham *et al.*, 1999; Storms *et al.*, 2008).

While this experimental set up allowed me to investigate the effects of deer exclusion on ticks and Lyme disease pathogen, it could have been improved if I had information on bank vole movement. Indeed, it would have been interesting to have a different type of fencing (e.g. different mesh size) that would have stopped bank voles from venturing out of their enclosures. I would have been able to see more clearly the effects of deer exclusion on Lyme disease and I might have seen larger differences in NIP and DIN. Additionally, it would have been interesting to have plots excluding deer in which I would have removed bank voles to see the repercussions on prevalence and DIN. I can hypothesise that, if I had such a set up, I should have found almost no ticks in exclosures, regardless of small mammal abundance and therefore, Lyme disease hazard should have been non-existent (Perkins *et al.*, 2006) (see results from Chapter 2).

3.7. Conclusion

In conclusion, high deer density had direct effects on vegetation structure and this was associated with a decrease in competent reservoir host abundance. Despite this, the density of infected nymphs was five times higher in high deer density plots due to a marked, positive effect, of deer on tick density. Although nymphal infection prevalence was lower in high deer density plots, suggesting a dilution effect of deer on *B. burgdorferi* s.l., their role as reproduction hosts, driving tick populations up, outweighed their role as dilution hosts. In terms of deer management, complete deer exclusion should result in a strong decrease in Lyme disease hazard but it is logistically unrealistic to completely remove deer from a large area in most situations. While this experimental design contrasted high deer densities with the absence of deer, I do not know the threshold of deer density that would result in similar effects observed with complete deer exclusion. Similarly, thresholds for positive effects of deer density on tick populations and negative effects on vegetation and small mammal communities are unknown and should be investigated further.

4. Unexpected consequences of the effect of food supplementation on Lyme disease hazard through a change in competent wood mouse density

4.1. Abstract

Pulsed availability of resources are common occurrences in nature and can strongly impact consumers' populations. These temporal oscillations can be crucial in the context of infectious diseases if they induce a change in reservoir host density or behaviour. This could be the case for Lyme disease, a vector-borne disease caused by bacteria from the *Borrelia burgdorferi* sensu lato complex and transmitted by Ixodid ticks. In this study, I used experimental supplementary feeding of wood mice (*Apodemus sylvaticus*), a competent reservoir host for *B. afzelii*, the most prevalent genospecies of the *B. burgdorferi* s.l. in Europe, to test the hypothesis that increased food supply increases Lyme disease hazard through a change in wood mouse abundance. Over two years, two out of four trapping grids were randomly supplemented with food for five months, which resulted in increased wood mouse abundance as confirmed by live trapping. Neither food supplementation nor wood mouse abundance affected individual tick burden on mice. For both years of the experiment, questing nymphs were surveyed the year following food supplementation and assessed for *B. burgdorferi* s.l. infection. Food supplementation the previous year *per se*, which reflects what would happened after a mast seeding event, had no effect on the density of questing nymphs (DON), nymphal infection prevalence (NIP) with *B. burgdorferi* s.l. nor the density of infected nymphs (DIN), a proxy for Lyme disease hazard. There was a positive correlation between wood mouse abundance the previous year and DON, while it did not affect NIP. I observed a positive correlation between wood mouse abundance the previous year and DIN for *B. garinii* and *B. valaisiana*, two genospecies associated with birds. I hypothesise that the role of wood mice in driving tick abundance in this context outweighs their potential role in diluting bird associated genospecies by diverting ticks from feeding on other competent birds. These results highlight how fluctuations of resources can affect host populations and lead to an increase in Lyme disease hazard.

4.2. Introduction

An individual's chances of successfully passing on its genes and thus, "succeeding" in life, depends on a plethora of factors ranging from adequate environmental conditions to the presence of predators, competitors and potential mates and species have evolved over millennia to be adapted to the environment in which they thrive. Despite species' adaptations to inhabit specific ecological niches, population growth is restrained by many factors, resources being one of the most limiting ones (Arrow *et al.*, 1995; Forbes *et al.*, 2014b; Huitu *et al.*, 2003; Sinclair *et al.*, 1985; Sinclair & Krebs, 2002; White, 1978). If resources were unlimited, populations would be expected to follow an exponential growth model (Meadows *et al.*, 1972) but this rarely happens in the natural world. Therefore, if population size is limited by the amount of resources available, what happens when resources vary?

Pulsed availability of resources are regular occurrences in nature and have been described in a wide range of systems. They are driven by a broad range of natural events such as salmon spawning (Wipfli *et al.*, 1999), mussel and cicada recruitment (Witman *et al.*, 2003; Yang, 2004) or El Niño oscillations (Stapp & Polis, 2003) and are a key driver of consumer populations size. One type of pulsed resource that has been extensively studied is mast seeding. This occurs when tree pollination booms in a synchronized manner, resulting in extremely high abundance of fruits available over a short period of time (Ostfeld & Keesing, 2000c; Schmidt, 2003). These mast seeding events are always followed by low fruit abundance years (Jones *et al.*, 1998), are unpredictable and strongly affect the populations of consumer species such as wild boar (*Sus scrofa*) and rodents (Clotfelter *et al.*, 2007; McShea, 2000; Ostfeld & Keesing, 2000c; Touzot *et al.*, 2020). One study showed that wild boar strongly responded to mast seeding events by increasing the proportion of females reproducing and decreasing age at first reproduction (Touzot *et al.*, 2020). Other studies in North America investigated the effect of acorn production on white footed mice (*Peromyscus leucopus*), deer mice (*Peromyscus maniculatus*) and grey squirrels (*Sciurus carolinensis*) and found an increase in overwintering survival, leading to a rise in densities in the spring following mast seeding years (Clotfelter *et al.*, 2007; Jones *et al.*, 1998; McShea, 2000; Ostfeld & Keesing, 2000c). A sudden increase in the availability of resources

can not only affect species' densities but also their behaviour (Cortés-Avizanda *et al.*, 2011; Hogstad, 2005; McDonald & Fuller, 2005; Watt *et al.*, 2000), body condition (Forbes *et al.*, 2015; Taylor *et al.*, 2005) and reproduction (Millon *et al.*, 2010; Montiglio *et al.*, 2014). Scrubs jays, for instance, were found to shift their breeding season earlier if they had access to more resources (Schoech *et al.*, 2004) while fed rattle snakes gained body mass faster, were in better condition and reproduced more compared to their unfed conspecifics (Taylor *et al.*, 2005).

Small mammals are often used to study the effect of changes in resources due to their fast life history strategy; they respond rapidly to a fluctuating environment due to their short generation time and rapid reproduction rate. Several studies have shown that a few months of food supplementation can increase population density by up to 50% (Desy & Batzli, 1989; Forbes *et al.*, 2015; Nie & Liu, 2005; Taitt & Krebs, 1981). The effects of food supplementation seem to be more marked when initial conditions are poor (Boutin, 1990). Mechanisms to explain the increase in population include reproductive females being more active, extension of the breeding period (Desy & Batzli, 1989; Perrin & Johnson, 1999) and increase in immigration (Perrin & Johnson, 1999; Taitt & Krebs, 1981) when food is provided. Offspring can also be in better body condition and infanticide can decrease (Rémy *et al.*, 2013). All of these mechanisms result in higher densities of individuals in good body condition.

Besides their essential ecological role as primary consumers, small mammals are important reservoirs for many parasites and pathogens (Holmberg *et al.*, 2003; Mills *et al.*, 1999; Reed *et al.*, 2003; Riehm *et al.*, 2011) and thus, changes in their populations could have dramatic impacts on disease prevalence and outbreaks (Davis *et al.*, 2004; Escutenaire *et al.*, 2000; Forbes *et al.*, 2015; Perez *et al.*, 2011; Tersago *et al.*, 2009; Tian *et al.*, 2015, 2017). In 2017, Tian *et al.* linked Hantaan virus incidence in China to a change in rodent abundance due to variations in rainfall and temperature affecting resource availability and winter survival of these reservoir hosts.

On the other hand, if the change in reservoir host density is due to an increase in resources, it is likely to lead to individuals in better condition (Forbes *et al.*, 2015; Taylor *et al.*, 2005) which could improve their immune responses (Becker *et al.*, 2015; Jessop *et al.*, 2012), thus resulting in lower parasitic burdens and pathogen prevalence (Anderson *et al.*, 2013; Jessop *et al.*, 2012; Michael & Bundy, 1991; Neve *et al.*, 2007; Niezen *et al.*, 2002; Oliver *et al.*, 2009; Osoro *et al.*, 2007). Additionally, these individuals may spend less time foraging and could allocate their energy towards grooming (Becker *et al.*, 2015), which could reduce ectoparasite burdens (Akinyi *et al.*, 2013; Shaw *et al.*, 2003) and infection rates (Taylor *et al.*, 2013; van Duijvendijk, 2016).

This study focused on the impact of food supplementation on a wood mouse (*Apodemus sylvaticus*) population and its cascading effects on the pathogen causing Lyme disease, an emerging vector-borne zoonosis. This bacterial disease, caused by spirochetes belonging to the genus *Borrelia burgdorferi* s.l., is transmitted primarily by the hard-bodied tick *Ixodes ricinus* in Europe (Stanek *et al.*, 2012). Larvae usually feed on small vertebrates such as birds and rodents and can become infected if they take a blood meal from an infected host or if they co-feed (i.e. in close spatial proximity) to another tick that is infected (Perez *et al.*, 2011; Randolph *et al.*, 1996; Voordouw *et al.*, 2015). Larvae moult into nymphs which then feed on a second host and can transmit the bacteria to humans if they feed on them. Nymphs moult into adult ticks and adult females must feed on a third host, usually a large mammal, before reproducing and laying eggs. Lyme disease hazard, defined at the density of nymphs infected with *B. burgdorferi* s.l., thus depends on the abundance of suitable tick hosts and competent reservoir hosts for each tick stage.

As well as being important hosts for larvae and nymphs, small mammals are competent reservoirs for *B. afzelii* (Hanincová *et al.*, 2003a; Kybicová *et al.*, 2008), the most prevalent *B. burgdorferi* s.l. genospecies in Europe (Strnad *et al.*, 2017). Birds maintain and transmit *B. garinii* and *B. valaisiana,* the next most abundance genospecies in Europe (Hanincová *et al.*, 2003b; Heylen *et al.*, 2014; Strnad *et al.*, 2017). The fourth and least abundant genospecies present in the United Kingdom, *B. burgdorferi* sensu stricto, is a generalist genospecies,

maintained by both birds and small mammals (Heylen *et al.*, 2014; Kurtenbach *et al.*, 1998a; Norte *et al.*, 2012; Richter *et al.*, 2000).

Therefore, small mammals can drive questing nymph abundance by providing suitable hosts for larval ticks (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2006) and can affect pathogen prevalence in questing nymphs by infecting larvae (Krawczyk *et al.*, 2020; Mysterud *et al.*, 2016; Vuong *et al.*, 2017). Thus, the abundance of small mammals is expected to influence Lyme disease hazard. Because resources are a key determinant of small mammal abundance, food resources might be a driver of Lyme disease hazard. Two cross-sectional studies in North America have shown a positive association between mast seeding events and white footed mouse density, which consequently impacted the density of nymphs infected with *B. burgdorferi* s.l. (Ostfeld *et al.*, 2001, 2006). However, the Lyme disease system in North America appears to be different than in Europe, where several genospecies occur simultaneously. Two studies conducted in the Netherlands (Krawczyk *et al.*, 2020; van Duijvendijk, 2016) showed that experimental food supplementation increased rodent density, which resulted in a rise in the density of nymphs infected with *B. afzelii* the following year, primarily due to a rise in nymph density. Interestingly, van Duijvendijk (2016) did not find any association between rodent density and pathogen prevalence while other studies in both Europe and North America have found that it was positively linked to rodent abundance the previous year (Krawczyk *et al.*, 2020; Mysterud *et al.*, 2016; Vuong *et al.*, 2017). The mechanisms for the links between resources, small mammal density and Lyme disease hazard are not yell fully understood and cannot be generalised. Therefore, with only two studies addressing this question in the complex European Lyme disease system, both from the Netherlands, there is a need for further studies in a contrasting location in terms of habitat, climate, host community composition and *B. burgdorferi* s.l. genospecies community.

This study had two goals: the first one was to assess the effects of food supplementation *per se* on Lyme disease hazard as it would reflect what would happen after a mast seeding event and include the entire host community. The second aim was to investigate the impacts of a change in a competent reservoir host abundance (wood mice) through food supplementation on individual tick burden on mice, the abundance of *I. ricinus* nymphs, *B. burgdorferi* s.l. prevalence

and the density of infected nymphs. I predicted that food supplementation should result in an increase in wood mouse density (Clotfelter *et al.*, 2007; Jones *et al.*, 1998; McShea, 2000; Ostfeld & Keesing, 2000b). Regarding individual tick burden, I predicted that tick burden on mice should be negatively correlated with wood mouse abundance (Levi *et al.*, 2016; Shaw *et al.*, 2003) because an increase in density means that ticks will be shared amongst more individuals, lowering individual burden (Kiffner *et al.*, 2011b). Similarly, I expected individual tick burden on mice to be lower in food supplemented grids as other hosts (birds, squirrels, bank voles) might be attracted and ticks will be shared amongst more individuals. Supplementary fed individual mice should be in better body condition (Forbes *et al.*, 2015) and thus, more able to groom ticks off themselves and reduce tick burdens (Gallivan *et al.*, 1995; Levin & Fish, 1998; Samuel, 1991), as well as enhanced immunity against parasites (Jessop *et al.*, 2012; Michael & Bundy, 1991; Neve *et al.*, 2007; Niezen *et al.*, 2002; Osoro *et al.*, 2007).

I set down three hypotheses for the effect of this change in wood mouse abundance and individual tick burden on Lyme disease hazard. For my first hypothesis (H1, Figure 4.1), I predicted that, under resource provision and increased host density, a larger proportion of larvae will successfully feed regardless of an increase in grooming activity (even if individual tick burden might be lower). Thus, I expected to observe a higher density of questing nymphs the following year (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2006). Regarding nymphal infection prevalence (NIP), I expected that a larger proportion of questing nymphs should be infected with *B. afzelii* as they would have been more likely to have fed on competent mice hosts as larvae (Krawczyk *et al.*, 2020; Mysterud *et al.*, 2016; Vuong *et al.*, 2017). I also expected to observe a lower NIP for bird associated genospecies (*B. garinii* and *B. valaisiana*) due to a dilution effect as the probability of larvae feeding on mice rather than birds should be higher (Krawczyk *et al.*, 2020). Regardless of NIP, the high density of nymphs should lead to a high density of infected nymphs (DIN).

On the other hand, if food supplementation leads to individuals in better body condition and with strong immune responses, I would expect to have fewer questing nymphs the following year, as they would have been unable to successfully feed (H2, Figure 4.1). Moreover, fewer nymphs should be infected

with *B. afzelii* relative to other genospecies. Overall, DIN should remain low due to the reduced number of questing nymphs.

When I compare my predictions to the control (Figure 4.1), I expected that mouse abundance would result in fewer larvae successfully feeding and decrease DON the following year. Regarding NIP, I predicted to have low to medium infection prevalence with *B. afzelii* and the other genospecies depending on the abundance of hosts in those grids. Regardless of NIP, DIN should be low due to the low density of questing nymphs. Because I did not record other host species that might have been affected by food supplementation (bids, squirrels, bank voles, see Löfgren *et al.*, 1996; McShea, 2000; Yoccoz *et al.*, 2001), I could not predict how they could affect Lyme disease hazard. If food supplementation attracted other hosts, I would expect to see an impact on DON. DON could either increase if more individuals spend time in the supplemented grids but also decrease if these individuals drop engorged larvae outside of supplemented grids. For NIP, I would expect to see a positive correlation between NIP for a specific genospecies and its associated host abundance. Investigating how NIP varied between food supplemented and control grids might give me an insight on how other host species might have been affected by food supplementation.

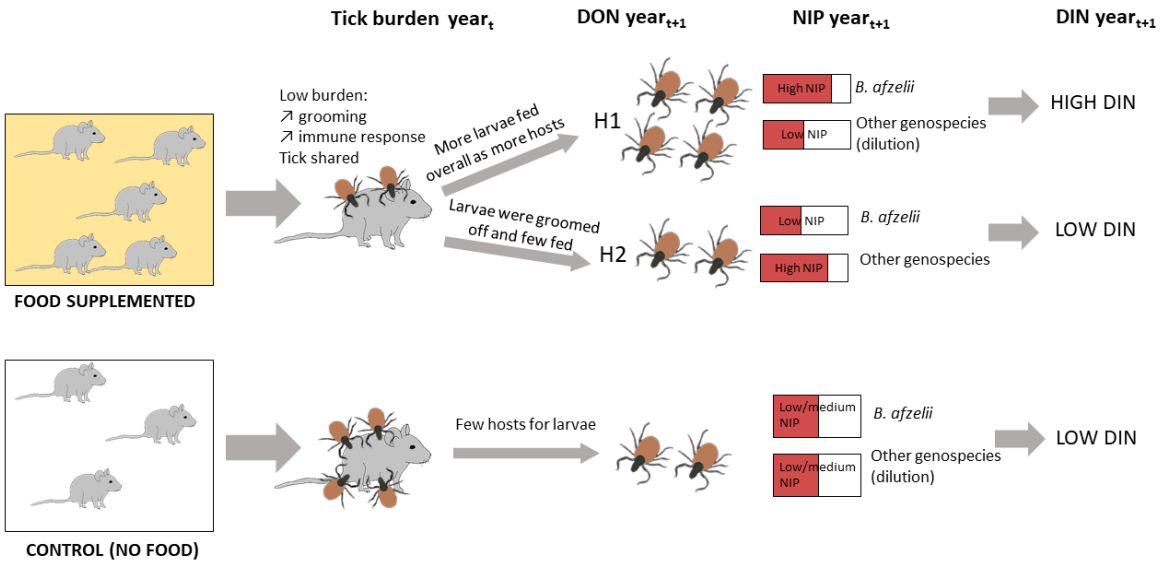


Figure 4.1: Conceptual figure to illustrate the hypotheses regarding the effect of food supplementation on tick burden the same year and the density of nymphs (DON), nymphal infection prevalence (NIP) and density of infected nymphs (DIN) the following year.

These predictions were tested using a replicated supplementary feeding experiment, combining field plots receiving food supplementation and controls, which did not receive supplementation. Live trapping of mice to assess mouse abundance and tick burdens as well as field surveys of questing nymphs were carried out to estimate tick density and *B. burgdorferi* s.l. prevalence.

4.3. Materials and methods

4.3.1. Field site and food supplementation protocol

The study site was located in an urban park within the city of Falkirk, Scotland (55.989°N, 3.765°W). Within this 95 Ha of mixed mature woodlands, four trapping grids of approximately 70m x70m were set up in 2017 by a research team from the University of Edinburgh. Grids were separated from one another by at least 20m to limit wood mice movements between them (Figure 4.2). In both 2017 and 2018, two grids were supplemented with food while the remaining two grids were controls and did not receive treatment. Grid 1 was supplemented in both years, grid 3 was a control in both years while grids 2 and 4 changed treatment group (Table 4.1)

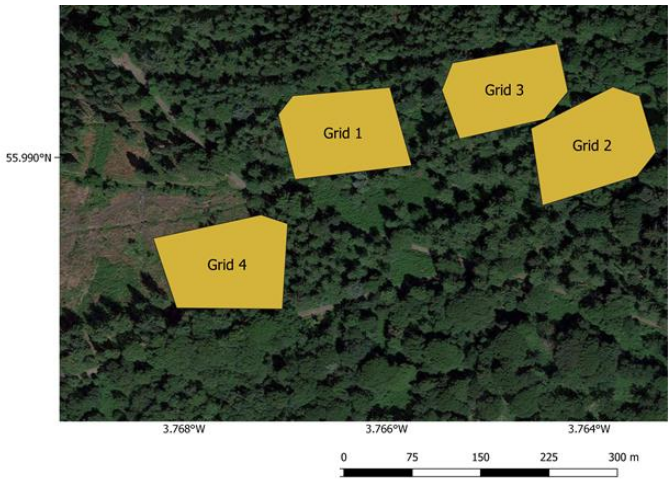


Figure 4.2: Aerial photograph showing the grid set up in Callendar wood, Falkirk, Scotland.

Table 4.1: Table summarising which grids received a food supplementation treatment in 2017 and 2018.

Year	Grid 1	Grid 2	Grid 3	Grid 4
2017	Supplemented	Control	Control	Supplemented
2018	Supplemented	Supplemented	Control	Control

Food supplemented grids were scattered evenly with sterilised Transbreed™ mouse chow pellets (2kg/1000m^2) three weeks before small mammal trapping began (i.e. June 28th in 2017 and June 22th in 2018) and twice weekly throughout the experiment (until November in 2017 and September in 2018) by a research team from the University of Edinburgh. Transbreed™ is a highly nutritious food used in captive mouse colonies and contains 20% of protein, 10.05% of fat, 38.48% of starch and micronutrients (Table 4.2).

Table 4.2: Nutrition content of the TransbreedTM food pellet using in field experiment. Full calculate analysis of nutritional contents can be found at www.sdsdiets.com/pdfs/TransBreed.pdf. Vit: Vitamin. Source: adapted from Sweeny, 2020.

Macronutrients (% by weight)			Micronutrients (quantity/kg)				
Protein	Fat	Starch	Zinc (Zn)	Iron (Fe)	Selenium (Se)	Vit A	Vit E
20.1	10.1	38.5	80.4 mg	216.0 mg	429.8 µg	18369 iu	267.4 iu

4.3.2. Wood mice (*Apodemus sylvaticus*) trapping

Small mammal trapping was conducted by a research team from the University of Edinburgh under the Home Office Regulations Project license 70/8543 for a separate study on the effect of food supplementation on intestinal parasites (Sweeny, 2020). In 2017, they trapped wood mice twice weekly for 2 non-consecutive nights between July 19th and November 17th (n=10,080 trap nights) while in 2018, trapping took place for three consecutive nights per week between July 3rd and September 6th (n=8,400 trap nights). Within each grid, 7x5 trapping stations were set up. A trapping station consisted of two Sherman live traps (H.B. Sherman 5x6.3x16.5 cm folding trap, Tallahassee, FL, USA) protected with plastic covers placed ten meters apart. This resulted in 70 traps per grid. Traps were baited using seeds, carrots and mealworms and cotton was provided as bedding. Traps were activated between 1600 hrs and 1800 hrs and checked the following morning. Sex, age, reproductive status, body mass and length were recorded for each wood mouse captured and wood mice were tagged with a subcutaneous microchip transponder (Friend Chip, AVID2028, Norco, CA, USA) if they weighed more than 13 grams. As part of the University of Edinburgh's study on intestinal parasites, at first capture, mice from all grids were alternatingly assigned within each sex to either control or anti-helminthic treatment groups. Mice from the drug

treatment groups were injected with a 2ml/g dose of Pyrantel pamoate (Strongid-P, 100 mg/kg) and Ivermectin (Eqvalan, 9.4mg/kg). Recaptured mice were treated with anti-helminthics every four weeks thereafter. Blood samples were taken at a maximum of once every four weeks via mandibular bleed at first capture and tail snip for subsequent captures (once per week maximum). All feeding ticks were counted around the head and ears and removed using tweezers and stored in 100% ethanol but tick life-stage and tick species was not recorded. If other species of small mammals were captured (bank voles *Myodes glareolus* and shrews *Sorex* spp.), they were immediately released at the capture site and captures were not recorded, because the University of Edinburgh's study on intestinal parasites focussed only on wood mice.

4.3.3. Questing *I. ricinus* nymphs surveys and *B. burgdorferi* s.l. assays

To test for the effect of food supplementation on tick density the following year, I surveyed and collected questing nymphs in 2018 and 2019 using a standard dragging method (Falco & Fish, 1992), where a 1m x 1m square of fleece blanket material was dragged over the ground for 10m. In 2018, questing nymphs were surveyed and collected at six occasions between the beginning of June and the end of September while in 2019, nymphs were collected five times between the end of March and mid-June. For each collection, twenty 10m-long linear random transects were conducted in each grid. Questing nymphs on the blanket were counted, collected in Eppendorf tube and stored at -20°C. Time of collection, dominant ground vegetation (bracken/fern, grass or moss), air temperature and relative humidity were recorded for each transect and all four grids were surveyed within the same three hours to minimise the potential effects of changing weather or time of day on tick activity. Vegetation height was measured at the beginning (0m), middle (5m) and end (10m) of each transect (Gilbert, 2010).

DNA from questing nymphal ticks was individually extracted using an ammonia extraction (Gern *et al.*, 2010, see Chapter 2 & 3) and tested for *B. burgdorferi* s.l. infection by qPCR (Heylen *et al.*, 2014, see Chapters 2 & 3). To identify the genospecies of *B. burgdorferi* s.l. in infected nymphs, positive samples were then processed using a nested PCR protocol (Rijpkema *et al.*, 1995, see Chapters 2 & 3) and sent to Edinburgh Genomics for Sanger sequencing. Three positive samples

could not be confirmed using the nested PCR so they were classified as *B. burgdorferi* s.l. and were excluded from the analyses that focussed on specific genospecies.

4.3.4. Statistical analyses

All statistical analyses were performed on the software R version 3.5.1 (R core team, 2013) using the packages glmmTMB (Brooks *et al.*, 2017; Magnusson *et al.*, 2019), lme4 (Bates, 2010) and MuMIn (Barton, 2019) packages.

I followed the same method for the analyses using generalized linear mixed effect models (GLMMs). First, I identified the appropriate distribution for each response variable and I assessed potential collinearity between explanatory variables by calculating variance inflation factors (VIFs). Variables with a VIF above 4 were not included in the models (Zuur *et al.*, 2009). Then, I tested each continuous variable in their linear and polynomial forms against the response variable and I used an F-test to assess model fit. I tested for overdispersion of the data by looking at the ratio between the residual deviance and the residual degrees of freedom (Harrison, 2014). If the ratio was above 1, it indicated overdispersion and an observation level random effect was added (Harrison, 2014, 2015).

If the response variable was a count, I used a GLMM with a Poisson distribution and I also fitted zero-inflation and hurdle GLMMs as the number of zeros might have been underestimated and I chose the best model based on AICc.

Model simplification was done using the dredge function from the MuMIn package (Barton, 2019) using AICc as the selection criterion (Brewer *et al.*, 2016; Burnham & Anderson, 2004; Johnson & Omland, 2004; Zuur *et al.*, 2009) and I calculated the conditional R^2(Johnson, 2014).

I did not have data on which individual mice received anti-helminthic treatment so I could not add it as a variable in the models. While several studies have demonstrated that such treatment does not affect Ixodid tick burdens on hosts (Sheele *et al.*, 2013, 2014; Taylor & Kenny, 1990), there is no clear evidence on the effect if could have on *Ixodes ricinus* ticks feeding on wood mice. Ticks feeding

on treated individuals could drop off and die after treatment, which would affect tick burden data.

4.3.4.1. The effects of food supplementation on wood mouse abundance

To investigate the effects of food supplementation on wood mice abundance in 2017 and 2018, I calculated the minimum number of wood mice alive (MNA), defined as the number of individuals caught in a capture session (t_i), plus those that were not caught at that time (t_i) but were caught both at a previous capture session (t_{i-1}) and subsequently (t_{i+1}) (Krebs, 1966). I used the minimum number alive as a proxy for wood mouse density and I will refer to it as wood mouse abundance hereafter.

I then used a GLMM with a Poisson distribution, using MNA per grid and per trapping day as my response variable. The full model included whether the grid was supplemented with food, trapping date in its quadratic form, as I expected mouse density to vary along the summer and because it was a better fit after assessing it using an F-test ($F_{1,184}$=14.0, $p<0.001$), year and an interaction between trapping date and food supplementation as fixed covariates. Grid number was added as a random effect.

4.3.4.2. The effects of food supplementation, wood mouse abundance and tick burden on mice the previous year on DON in the spring

For this analysis, I used the first observation for each individual as well as any recaptures that occurred more than five days after the previous capture as tick re-infestation stabilised after five days (Appendix C, S1).

While this study really focussed on the effects of food supplementation on one specific host (wood mice), I also wanted to investigate the effects of food supplementation *per se* on Lyme disease hazard as it would reflect what would happen after a mast seeding even and include other hosts that were not recorded during this study (i.e. birds, bank voles, squirrels). Thus, I decided to run two separate analyses: the first model focussed on the impact of food supplementation *per se* on various parameters while the second one assessed the effects of wood mouse abundance on these same parameters.

To investigate the impact of food supplementation on tick burden per individual wood mice, I ran a GLMM with a Poisson distribution with the response variable being the number of ticks feeding on wood mice. The first model included food supplementation the same year (supplemented or control), sex, age, year, trapping date and the interaction between date and food supplementation as fixed covariates. Random effects of grid number, microchip ID and an observation level random effect were included.

In a second model, I used mouse abundance instead of food supplementation and the full model included mouse abundance, sex, age, year, trapping date and the interaction between date and mouse abundance as explanatory variables. Random effects of grid number, microchip ID and an observation level random effect.

I also used non-parametric Mann-Whitney tests to compare tick burden on mice between individuals that moved between grids and those who were always captured in the same grid. I used a Mann-Whitney test because the samples were independent and not normally distributed.

4.3.4.3. The effects of food supplementation, wood mouse abundance and tick burden on questing nymph abundance in the spring

In order to investigate how food supplementation, mouse abundance and tick burden on mice the previous year affected the density of questing nymphs in the spring (from March to June), I used two GLMMs with a Poisson distribution and my response variable was the number of questing nymphs collected per 10m transect in the spring.

The first model included food supplementation the previous year (food supplemented or control), date in its quadratic form ($F_{1,583}$=42.8, $p<0.001$), vegetation height, humidity (as it can affect tick questing behaviour), year and ground vegetation type as fixed explanatory variables. Random effects of grid number and an observation level random were included.

Instead of food supplementation, the second model included wood mouse abundance the previous year in its quadratic form ($F_{1,583}$=23.6, $p<0.001$), mean individual tick burden per grid the previous year, date in its quadratic form

($F_{1,583}$=42.8, p<0.001), vegetation height, humidity, year, ground vegetation and the interaction between wood mouse abundance and tick burden fixed explanatory variables. I added this interaction as the effect of wood mouse abundance on questing tick abundance the following spring is likely to be dependent on individual tick burden on mice. For individual tick burden, I used the first observation for each individual as well as any recaptures that occurred more than five days after the previous capture, as described above, and I averaged individual burden per grid and per year. To simplify this analysis, I separated tick burden into three categories (low, medium, high) using the interquartile range: low burden from the minimum number of ticks collected on a mouse to the 1st quartile, medium burden from 1st to 3rd quartiles and high burden when higher than 3rd quartile[2]. Random effects of grid number and an observation level random effect were included. Temperature was not included because it was correlated with date (VIF=4.2). Continuous variables were scaled.

4.3.4.4. The effects of food supplementation, wood mouse abundance and tick burden on mice the previous year on NIP in the spring

After testing the questing nymphs collected, none were infected with *B. afzelii* so the analysis focusing on the effects of an increase in mouse abundance on this rodent associated genospecies could not be performed.

Regarding the analysis focussing on samples infected with *B. garinii* and *B. valaisiana*, the response variable was the proportion of questing nymphs infected per grid on each sampling event (6 prevalence estimates in 2018 and 5 in 2019). I used two GLMMs with a binomial distribution and logit link. The first model included food supplementation the previous year, date during tick collection and year as fixed variables. Random effects of grid number and an observation level random effect were included.

The second model included wood mouse abundance the previous year, the average individual tick burden (using the first observation for each individual as well as any recaptures that occurred more than five days after the previous capture and

[2] This analysis was also done keeping individual burden in its continuous form and gave the same results.

averaging individual burden per grid and per year), year, date during tick collection and the interaction between wood mouse abundance and individual tick burden as fixed effects. Random effects of grid number and an observation level random effect were included. Continuous variables were scaled.

4.3.4.5. The effects of food supplementation, wood mouse abundance and tick burden on mice the previous year on DIN with *B. garinii* and *B. valaisiana* in the spring

To investigate the factors affecting the density of nymphs infected with *B. garinii* and *B. valaisiana*, I used two zero-inflation GLMMs with a Poisson distribution. The response variable was the number of nymphs infected per $1000m^{-2}$ in the spring and, as this variable was averaged at the grid level for each collection day. I used an offset for the area to transform the values to integers (i.e. if 6.5 nymphs were infected/$1000m^{-2}$, it was transformed as 7 nymphs infected/$1076m^{-2}$, Zuur *et al.*, 2009). The first model included food supplementation the previous year, date during tick collection, relative humidity and year as fixed variables. Random effects of grid number and an observation level random effect were included.

The second model included wood mouse abundance the previous year in its quadratic form ($F_{1,25}$=4.5, p=0.04), the average individual tick burden (using the first observation for each individual as well as any recaptures that occurred more than five days after the previous capture and averaging individual burden per grid and per year), year, date of tick collection, relative humidity and the interaction between wood mouse abundance and individual tick burden as fixed effects. Random effects of grid number and an observation level random effect were included. There was a correlation between temperature (VIF=6.68), vegetation height (VIF=4.07) and year and thus, temperature and vegetation height were not included in the models. Continuous variables were scaled.

4.4. Results

During data exploration, I found that 25 individuals (out of 169) in 2017 and 19 individuals (out of 79) in 2018 were trapped in more than one grid within a trapping season. Therefore, I could not assign them to a grid and these individuals were excluded from the analyses. Tick burdens were compared among individuals which moved between grids and those which did not and were similar (for males: Mann-Whitney U= 11548, n_1=107, n_2=204, p=0.39 two tailed. For females: Mann-Whitney U= 8721, n_1=84, n_2=185, p=0.10 two tailed), supporting that including these mice would not have affected the results (Figure 4.3).

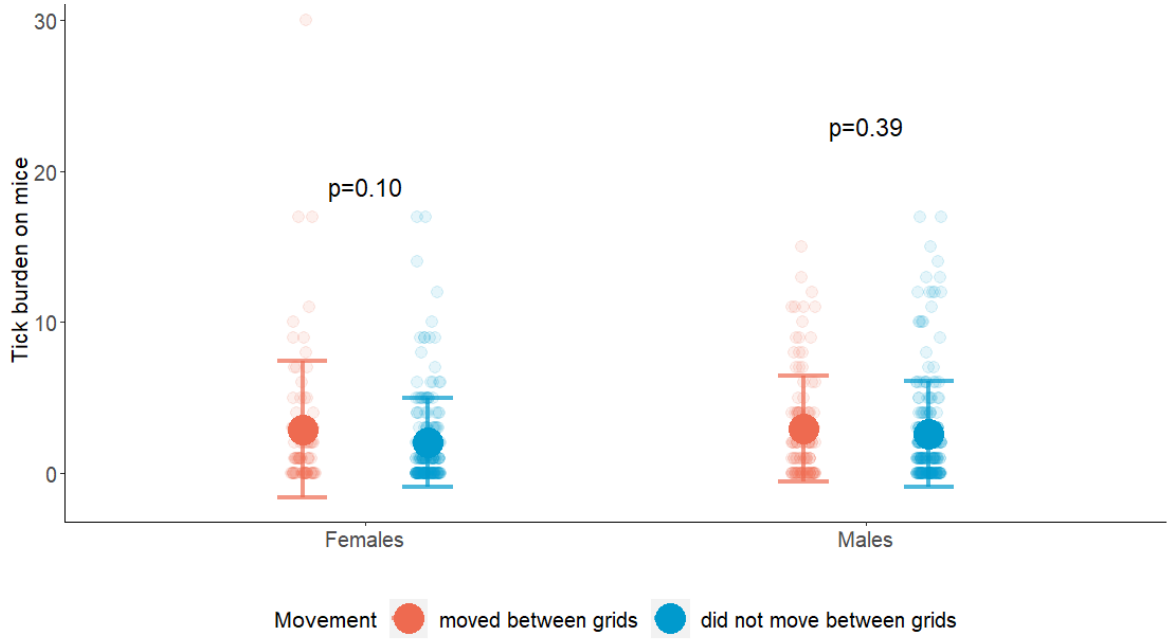


Figure 4.3: Mean individual tick burden for female (red) and male (blue) wood mice depending on whether these individuals were trapped in different grid or if they were consistently captured in the same grid. P-values represent results from Mann-Whitney tests comparing individual tick burden between individuals that moved between grids and those that did not for males (Mann-Whitney U= 11548, n_1=107, n_2=204, p=0.39) and for females (Mann-Whitney U= 8721, n_1=84, n_2=185, p=0.10).

In total, 144 individual mice were captured in 2017 and 60 in 2018 (excluding the ones that moved between grids). In 2017, 72 females and 72 males were caught, 106 of which were adults, 36 subadults and 2 juveniles. In 2018, 26 mice were females and 34 males with 43 adults and 17 subadults. On average, 9.3 mice were captured per 100TN (SD=4.8) in food supplemented grids in 2017 and 7.7/100TN (SD=3.3) in 2018 while in control grids, 7.4/100TN (SD=3.7) were captured in 2017 and 5.9/100TN (SD=3.6) in 2018.

4.4.1. The effects of food supplementation on wood mouse abundance

The simplified model explained 57% of the variance and included plot treatment (food supplemented or control) and trapping date in its quadratic form (Table 4.3). The minimum number of mice alive (MNA) was higher in food supplemented grids (predicted MNA=7.65, 95%CI: 6.00-9.76) compared to control grids (predicted MNA=3.55, 95%CI: 2.78-4.53) and varied in the season (peaking in September/early October; Figure 4.4 & Table 4.3). Year was dropped from the model during selection.

Table 4.3: Results from the selected model to explain what causes variations in the minimum number of wood mice alive. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	1.36	0.13			
Treatment: supplemented	0.77	0.08	87.8	44.9	1
Date	0.25	0.04	35.6	19.8	2
Date^2	-0.14	0.03	27.9	14.9	1

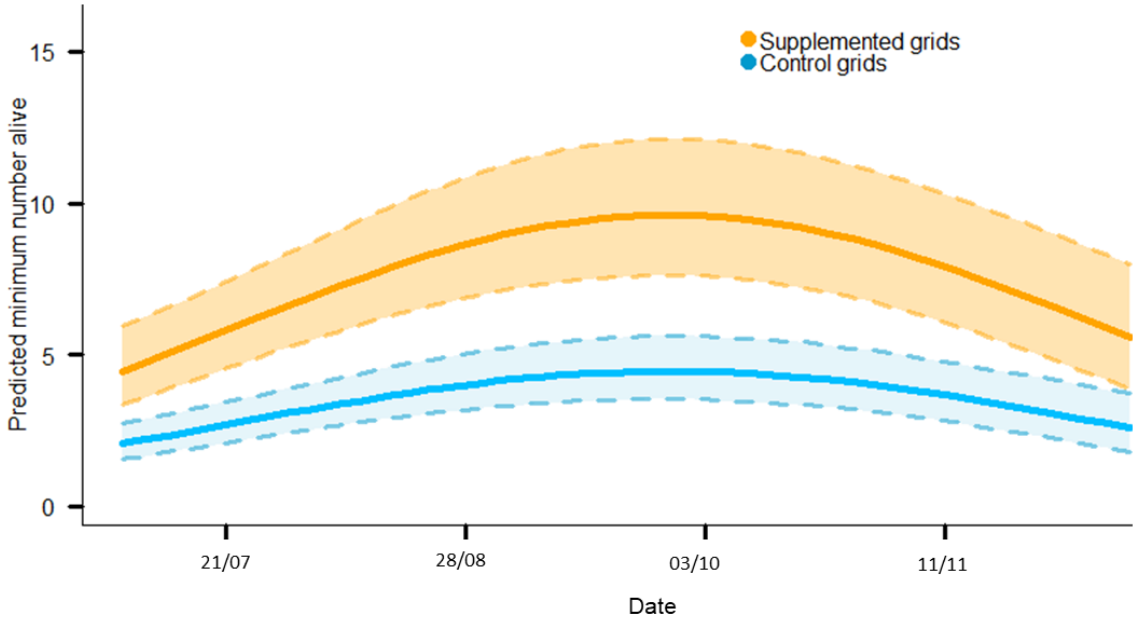


Figure 4.4: Predicted wood mouse abundance (MNA) depending on the date in control (blue) and food supplemented (orange) grids. Shaded areas represent the 95% CIs.

4.4.2. The effects of food supplementation and wood mouse abundance on individual tick burden on mice

Neither food supplementation nor wood mouse abundance had a clear effect on tick burden (Table 4.4 & Table 4.5).

Table 4.4: Selection of models of tick burden on individual mice including food supplementation: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the best fit model and did not include food supplementation (Food).

Age	Date	Sex	Food	Year	Food * date	df	log-Lik	AICc	ΔAICc
+	+	+	-	+	-	8	-743.0	1502.3	0
+	+	+	+	+	-	9	-742.1	1502.7	0.4
-	+	+	-	+	-	7	-744.9	1504.1	1.8
+	+	-	-	+	-	7	-745.1	1504.5	2.2
-	+	+	+	+	-	8	-744.1	1504.5	2.2
+	+	+	+	+	+	10	-742.1	1504.7	2.4
+	+	-	+	+	-	8	-744.3	1505.0	2.7

Table 4.5: Selection of models of tick burden on individual mice including wood mouse abundance: models with a ΔAICc<3: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the best fit model and did not include wood mouse abundance.

Age	Date	Sex	Mouse density	Year	Mouse density * date	df	log-Lik	AICc	ΔAICc
+	+	+	-	+	-	8	-743.0	1502.3	0
-	+	+	-	+	-	7	-744.9	1504.1	1.8
+	+	+	+	+	-	9	-742.9	1504.2	1.9
+	+	-	-	+	-	7	-745.1	1504.5	2.2
+	+	+	+	+	+	10	-742.4	1505.3	3.0

4.4.3. The effects of food supplementation, wood mouse abundance and tick burden on mice the previous year on questing nymph abundance (DON) in the spring

A total of 1,146 questing nymphs were collected (grid 1: n=171, grid 2: n=301, grid 3: n=295, grid 4: n=379) over two years of survey. On average, 1.2 (SD=1.6) nymphs were collected per $10m^{-2}$ in grids supplemented with food the previous year (2018: mean=0.7, SD=1.1 ; 2019: mean=1.8, SD=1.8) compared to 1.3 (SD=1.7) in control grids (2018: mean=0.8, SD=1.3 ; 2019: mean=2.0, SD=1.9).

Food supplementation *per se* did not affect questing nymphs density the following spring, as the variable did not improve model fit and the final selected model did not include food supplementation (Table 4.6).

Table 4.6: Selection of models of questing nymphs density including food supplementation: models with a ΔAICc<3: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the best fit model and did not include food supplementation the previous year. Vege height: vegetation height, Ground vege: ground vegetation, RH: relative humidity, Food: Food supplementation.

Vege height	Ground vege	$Date^2$	RH	Food $year_{t-1}$	Year	df	log-Lik	AICc	ΔAICc
+	-	+	+	-	+	8	-908.0	1832.2	0
+	-	+	+	-	-	7	-909.6	1833.3	1.1
+	-	+	+	+	+	9	-907.7	1833.7	1.5
+	-	+	-	-	+	7	-910.4	1835.0	2.8
+	-	+	+	+	-	8	-909.4	1835.1	2.9

Questing nymph density in the spring was influenced by wood mouse abundance and tick burden on mice the previous year and the simplified model included vegetation height, date of tick collection, relative humidity, wood mouse abundance and tick burden on mice the previous year (Table 4.7).

There was a positive correlation between wood mouse abundance the previous year and the density of questing nymphs (DON) until a plateau was reached at high mouse density (Table 4.7 & Figure 4.5-A). Regarding tick burden, DON was higher for medium tick burden the previous year (predicted DON=1.73, 95%CI: 1.29-2.33) compared to low (predicted DON=0.78, 95%CI: 0.56-1.10) and high (predicted DON=0.66, 95%CI: 0.44-0.98) tick burdens on mice (Table 4.7 & Figure 4.5-B).

I also found negative correlations between DON, date and vegetation height while there was a positive association with humidity (Table 4.7) while ground vegetation and year did not seem to affect tick abundance as they were dropped from the model. Overall, the model explained 34% of the variance.

Table 4.7: Results from the selected model to explain what causes variations in questing nymph abundance in the spring. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Intercept	0.14	0.18			
Vegetation height	-0.29	0.07	14.6	8.3	1
Date	-0.17	0.07	29.7	16.8	2
Date^2	-0.37	0.07	31.1	16.5	1
Humidity	0.09	0.05	1.9	1.9	1
Tick burden year$_{t-1}$			6.6	5.3	2
Low	0.17	0.21			
Medium	0.97	0.24			
Mouse density year$_{t-1}$	0.36	0.11	12.0	8.0	2
Mouse density year$_{t-1}^2$	-0.30	0.12	3.8	2.9	1

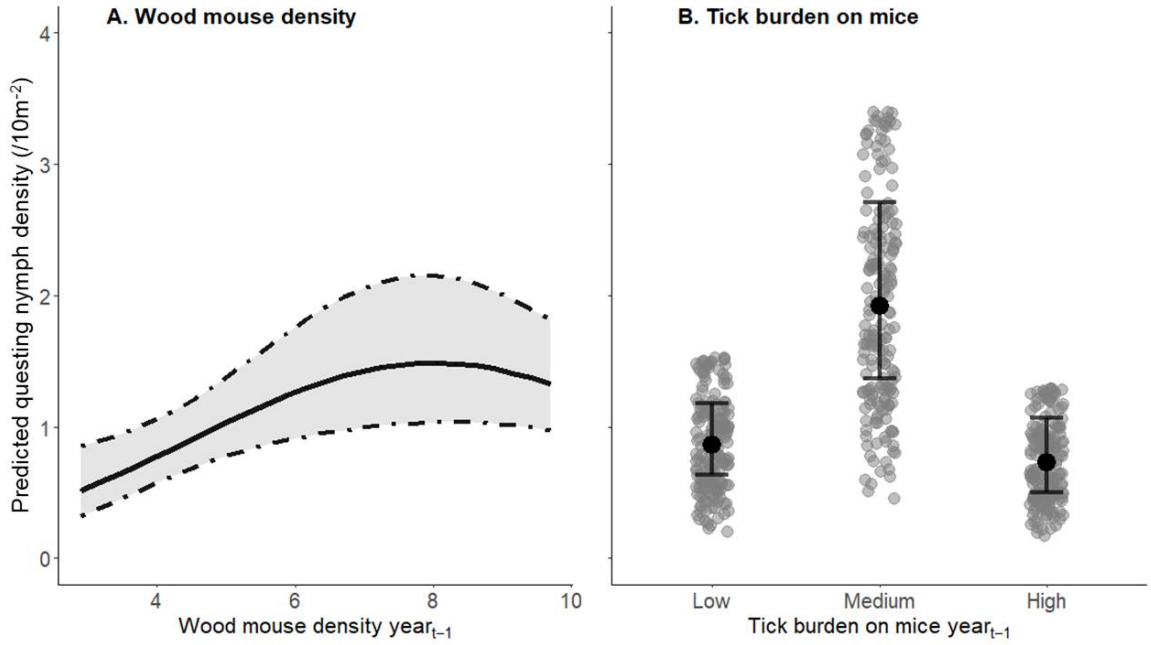


Figure 4.5: Predicted density of questing nymphs/10m in the spring depending on (A) wood mouse abundance (minimum number of wood mice alive) and (B) tick burden on mice the previous year. The shaded area and error bars represent the 95% CIs.

4.4.4. The effects of food supplementation, wood mouse abundance and tick burden on mice the previous year on nymphal infection prevalence (NIP) in the spring

All the questing nymphs collected (n=1,146) were screened for presence of *B. burgdorferi* s.l. over the two years of data collection. Of these, 30 (2.6%, 95%CI: 1.8-3.7) were found to be infected with *B. burgdorferi* s.l., (Table 4.8). The dominant genospecies in the study area was *B. garinii* (53% of positive sample,

n=16/30), followed by *B. valaisiana* (37%, n=11/30). No samples were found to be infected with *B. afzelii* or *B. burgdorferi* s.s. and genospecies could not be identified for 3/30 positive samples (10%) (Figure 4.6).

Table 4.8: Table summarizing the proportion of questing ticks infected with *Borrelia burgdorferi* s.l. (NIP) as well as the number of questing nymphs infected per 1000m^{-2} (DIN) in each of the four grids in 2018 and 2019.

Grid	Food suppl. year$_{t-1}$	NIP (%) (no. positive/no. tested)	DIN	Food suppl. year$_{t-1}$	NIP (%) (no. positive/no. tested)	DIN
	2018			**2019**		
1	Suppl.	0 (0/46)	0	Suppl.	1.6 (2/125)	2.0
2	Control	6.1 (4/66)	3.23	Suppl.	3.4 (8/235)	8.0
3	Control	0.7 (1/137)	0.79	Control	5.7 (9/158)	9.0
4	Suppl.	1.4 (2/138)	1.38	Control	1.7 (4/241)	4.0

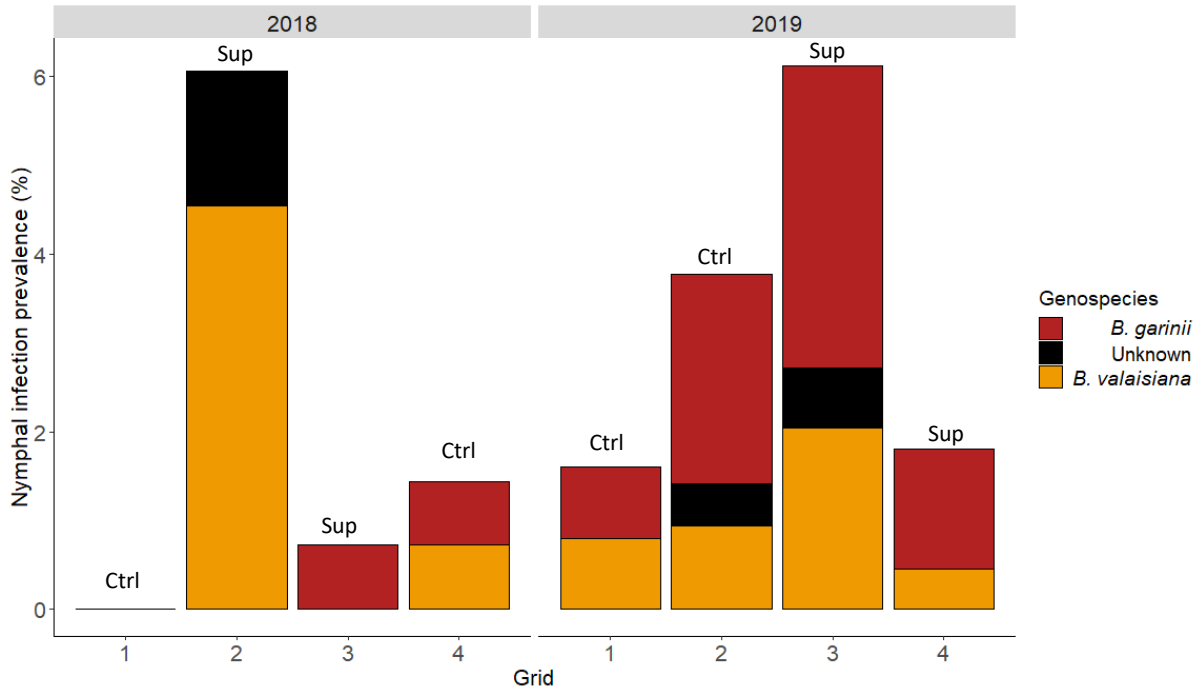


Figure 4.6: Nymphal infection prevalence (%) with *B. garinii* (red), *B. valaisiana* (orange) and unknown genospecies (black) in all four trapping grids in 2018 and 2019. No questing nymphs were infected with *B. afzelii* or *B. burgdorferi* s.s. Sup refers to grids that were supplemented with food the previous year and Ctrl to control grids.

Although I predicted that food supplementation should lead to either higher (H1) or lower (H2) prevalence of *B. afzelii* relative to controls, no questing ticks were found to be infected with *B. afzelii* and thus, these hypotheses could not be tested.

Neither food supplementation, wood mouse abundance nor individual tick burden (reflecting how many ticks fed on mice) influenced the nymphal infection prevalence (NIP) of bird associated genospecies (i.e. *B. garinii* and *B. valaisiana*) the following spring. (Table 4.9 & Table 4.10).

Table 4.9 Selection of models of NIP including food supplementation: models with a ΔAICc<3: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the best fit model and did not include food supplementation the previous year.

Date	Year	Food suppl. $year_{t-1}$	df	log-Lik	AICc	ΔAICc
+	-	-	3	-29.8	66.7	0
+	+	-	4	-29.4	68.6	1.9
+	-	+	4	-29.6	69.0	2.3

Table 4.10: Selection of models of NIP including wood mouse abundance and tick burden on mice: models with a ΔAICc<3: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the best fit model and did not include wood mouse abundance nor tick burden on mice.

Date	Mouse density $year_{t-1}$	Tick burden	Year	Mouse density $year_{t-1}$ * Tick burden	df	log-Lik	AICc	ΔAICc
+	-	-	-	-	4	-29.8	69.3	0
+	-	+	-	-	5	-29.2	71.1	1.7
+	-	-	+	-	5	-29.4	71.5	2.1
-	-	-	-	-	3	-32.4	71.7	2.4
+	+	-	-	-	5	-29.7	72.2	2.9

4.4.5. The effects of food supplementation, wood mouse abundance and tick burden on mice the previous year on the density of infected nymphs (DIN) in the spring

There was no association between the density of questing nymphs infected with bird associated genospecies (DIN) in the spring and food supplementation the previous year (Table 4.11).

Table 4.11: Selection of models of DIN including food supplementation: models with a ΔAICc<3: this table is showing the models with a ΔAICc below 3 obtained with the dredge function along their log-likelihood, AICc and ΔAICc values. For each of the fixed covariates, the + sign shows models in which this variable was kept while the - sign shows variables that were not retained. The first model, shaded in grey, was the best fit model and did not include food supplementation the previous year.

Date	Relative humidity	Year	Food suppl. $year_{t-1}$	df	log-Lik	AICc	ΔAICc
-	+	+	-	6	-56.7	129.3	0
+	-	-	-	5	-58.5	129.8	0.5
-	-	+	-	5	-58.7	130.1	0.8
+	-	-	+	6	-57.6	131.3	1.9
+	+	-	-	6	-57.7	131.5	2.2
-	+	+	+	7	-56.0	131.6	2.3
+	+	-	+	7	-56.1	131.9	2.5
+	-	+	-	6	-58.0	132.1	2.8

Regarding the effect of wood mouse abundance and the average tick burden on mice the previous year, the simplified model included relative humidity and wood mouse abundance as predictors. DIN was negatively correlated with wood mouse abundance at low density while there was a positive correlation from medium to high density of mice (Table 4.12 & Figure 4.7). DIN was also positively correlated with humidity while it seemed that date, year and tick burden on mice the previous year did not have any effect on DIN.

Table 4.12: Results from the selected model to explain what causes variations in the density of questing ticks infected with *B. garinii* and *B. valaisiana*. ΔAICc and Δlog-Lik indicate the increase in AICc and log-likelihood after dropping each variable from the model. Δdf represents the change in degrees of freedom.

	Estimate (Log)	Std Error	ΔAICc	Δlog-Lik	Δdf
Conditional model					
Intercept	-5.85	0.32			
Relative humidity	0.65	0.17	8.6	5.4	1
Mouse density $year_{t-1}$	-0.42	0.21	6.5	5.3	2
Mouse density $year_{t-1}^2$	0.81	0.22	6.3	4.2	1
Zero-inflation model					
Intercept	-0.14	0.41			

Figure 4.7: Predicted density of questing nymphs infected with *B. garinii* and *B. valaisiana* per 1000m^{-2} in the spring depending on wood mouse abundance (minimum number alive) the previous year. Shaded area represents the 95% CI.

4.5. Discussion

While the primary goal of this study was to investigate the effects of a change in a competent reservoir host density through food supplementation, using wood mice as a model, on Lyme disease hazard, I was also interested in the effects of food supplementation *per se* on Lyme disease hazard as it would reflect what would happen after a mast seeding event. Food supplementation itself had no effect on the density of nymphs (DON), nymphal infection prevalence (NIP), the density of infected nymphs (DIN) the following spring, nor on individual tick burden on mice the same year (Table 4.13). However, food supplementation increased the number of individual wood mice in the same year of treatment and, furthermore, increased wood mouse abundance was associated with increased DON and DIN the following spring (Table 4.13). Regarding DON, it was also affected by tick burden on wood mice and mice with medium burden led to higher DON the following spring. (Table 4.13).

Table 4.13: Table summarizing the results for the effect of food supplementation on wood mouse abundance, of food supplementation and wood mouse abundance the same year on tick burden on mice and of food supplementation and wood mouse abundance the previous year on DON, NIP and DIN in the spring.

Response variable	Explanatory	Effect	Figure/ Table
Minimum number alive	Food suppl.	Increased mouse abundance in supplemented grids	Figure 4.4
Individual tick burden	Food suppl. $year_t$ Mouse density $year_t$	No effect No effect	Table 4.4 Table 4.5
DON	Food suppl. $year_{t-1}$ Mouse density and burden $year_{t-1}$	No effect Positive correlation between mouse abundance and DON Medium tick burden led to high DON	Table 4.6 Figure 4.5
NIP *B. garinii* and *B. valaisiana*	Food suppl. $year_{t-1}$ Mouse density and burden $year_{t-1}$	No effect No effect	Table 4.9 Table 4.10
DIN *B. garinii* and *B. valaisiana*	Food suppl. $year_{t-1}$ Mouse density and burden $year_{t-1}$	No effect Positive correlation between mouse abundance and DIN, no effect of burden	Table 4.11 Figure 4.7

As seen in other studies, food supplementation successfully increased wood mouse abundance (Figure 4.4) (Desy & Batzli, 1989; Nie & Liu, 2005; Taitt & Krebs, 1981). Because grid treatment changed between years, I assume that the changes in density I observed were due to food supplementation. For all grids, there was an increase in wood mouse abundance throughout summer (July to September, Figure 4.4) followed by a decline from October onwards, regardless of grid treatment, which follows the typical pattern of increasing numbers of wood mice over the summer due to their offspring production (Fernandez *et al.*, 1996; Kikkawa, 1964). Although bank vole abundance was not recorded during this study, they are competent reservoir hosts for *B. afzelii* (Hanincová *et al.*, 2003a; Kurtenbach *et al.*, 1994) and play an important role in the Lyme disease epidemiological cycle. Several studies have shown that food supplementation increased bank vole density (Löfgren *et al.*, 1996; Yoccoz *et al.*, 2001) so I would expect their abundance to increase in food supplemented grids similarly to what was observed for wood mice.

After confirming the positive effect of food supplementation on this wood mouse population, I investigated the effects on individual tick burden and predicted that

mice caught in supplemented grids should have lower individual tick burdens. This was based on previous studies which have shown that food supplementation improves body condition (Forbes *et al.*, 2015; Forbes *et al.*, 2014a; Sweeny, 2020) and increases grooming activity (Gallivan *et al.*, 1995; Levin & Fish, 1998; Samuel, 1991), which could result in lower burdens (Levi *et al.*, 2016; Shaw *et al.*, 2003) as more individuals would deplete the pool of questing ticks, resulting in lower per capita burdens (Kiffner *et al.*, 2011b). Furthermore, larvae should be shared among more wood mice, further reducing tick burden on individual mice (Kiffner *et al.*, 2011b). Contrary to my predictions, neither food supplementation nor wood mouse abundance were associated with individual tick burden (Table 4.4). A possible reason for this is that the difference in wood mouse abundance between grids was not large enough to see an effect on burden and food supplementation might have not resulted in individuals in better body condition. Life-stage of feeding ticks was not recorded and it would have been interesting to separate larvae and nymphs in the analysis as I might have detected an effect. However, another study conducted in Scotland showed that 99% of ticks feeding on small mammals were larvae (James, 2010) so it is unlikely that these results would have changed.

From my first hypothesis (H1, Figure 4.1), I expected that, if food supplementation increases wood mouse abundance, a larger proportion of the questing larvae population would successfully feed (regardless of an increase in grooming activity) because their chances of encountering a suitable host would be higher, resulting in higher DON the following year, as found by other studies (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2006). If the grooming activity increased (H2, Figure 4.1) and fewer ticks successfully fed, I would expect a decrease in DON the following year (Akinyi *et al.*, 2013; Shaw *et al.*, 2003). Food supplementation *per se* did not affect DON the following spring (Table 4.6). There is no reason to think that food supplementation did not attract other hosts so these results might suggest that other host species (e.g. birds, squirrels) might have picked up larvae while feeding on the supplemented grids but dropped them off elsewhere.

Mouse density was positively correlated with DON the following spring (Figure 4.5), which supports the first hypothesis. These results are in line to those of Krawczyk *et al.* (2020), who conducted a similar experiment and found a positive effect of

rodent density on DON the following year while they did not find an effect of food supplementation per se on DON.

As expected, wood mice harbouring a medium tick burden resulted in higher DON the following spring (predicted DON=1.73, 95%CI: 1.29-2.33) compared to those with a low individual burden (predicted DON=0.78, 95%CI: 0.56-1.10, Figure 4.5). Interestingly, I also found that grids with mice supporting the highest tick burden resulted in the lowest DON the following spring (predicted DON=0.66, 95%CI: 0.44-0.98, Figure 4.5), which goes against the predictions. This could occur if grids with lower number of mice had mice with high burden of ticks but I did not test that. Alternatively, a mismatch between tick burden and DON the following year could occur if different species of ticks are causing high tick burdens but are not counted during questing nymphs survey. Although *Ixodes ricinus* is the main tick species feeding on small mammals, other species such as *I. trianguliceps* also take blood meals from these hosts. One study conducted in Scotland found that 91% of larvae found on rodents were *I. ricinus* and 4% were *I. trianguliceps* (James *et al.*, 2011) while in Norway, 73% of larvae feeding of wood mice were *I. ricinus* and 27% were *I. trianguliceps* (Mysterud *et al.*, 2015). As only *I. ricinus* ticks are collected with the dragging method (James, 2010; Randolph, 1975), this could explain the association observed between high tick burden and low DON.

Out of the 1,146 questing nymphs tested, none were infected with *B. afzelii*. This came as a surprise as two other studies conducted simultaneously in Scotland showed *B. afzelii* to be the dominant genospecies (see Chapters 2 & 3), and 3 previous studies have also shown *B. afzelii* to be the dominant genospecies in Scotland (45-48% of infected nymphs) (James *et al.*, 2013, 2014; Millins *et al.*, 2016). Therefore, I could not test my hypothesis that wood mouse density should be associated with *B. afzelii* prevalence the following year.

Instead, when focussing on nymphal infection prevalence (NIP) with *B. garinii* and *B. valaisiana*, two genospecies associated with bird species (Hanincová *et al.*, 2003b; James *et al.*, 2011; Taragel'ová *et al.*, 2008), I expected to observe a negative association between NIP and wood mouse abundance (H1), because a higher proportion of ticks should be feeding on wood mice instead of birds. This effect has been demonstrated by Krawczyk *et al.* (2020), who found a negative

association between rodent density and *B. garinii* prevalence in questing nymphs the following year, which is consistent with a dilution effect. However, I did not see an effect of either individual tick burden on mice or mouse density the previous year on NIP for *B. garinii* and *B. valaisiana* in the spring. One possible reason is that ground-feeding birds could also have been attracted by the supplementary food, which was scattered on the ground, as previously shown by Källander (1981). However, if birds had been attracted, I would expect to have a higher NIP for *B. garinii* and *B. valaisiana* in food supplemented grids compared to controls, which was not the case here. Because other hosts were not quantified here, it is difficult to understand this lack of effect and thus, future supplementary feeding studies should also consider the potential effects on other host species. In their study, Krawczyk *et al.* (2020) used feeding stations that prevented access by birds and squirrels, which could explain why they observed a strong dilution effect.

Based on my initial predictions, I expected to see either a positive association between mouse abundance or higher tick burdens and DIN (H1, Figure 4.1) due to higher DON and/or NIP or a decrease in DIN (H2, Figure 4.1) if food supplementation led to lower DON the following year due to improved body condition and grooming and/or ticks being shared between more mice. Intriguingly, I found some support for both, as I observed a quadratic relationship between mouse abundance the previous year and DIN for *B. garinii* and *B. valaisiana* (Figure 4.7). There was an initial decrease in DIN at low to medium mouse abundance followed by a strong, positive effect of mouse abundance on DIN. This initial decline suggests that, when mouse abundance is low, ticks might feed on other hosts species while the positive effect on DIN is probably due to the effect of wood mice in driving tick densities. Indeed, if this increase in DIN was due to birds being attracted to supplemented grids, I should have observed a higher DIN for *B. garinii* and *B. valaisiana* in supplemented grids, which was not the case (Table 4.11) and these results go against previous finding (Takumi *et al.*, 2019). In their cross-sectional survey, Takumi *et al.* found a negative association between wood mouse abundance and DIN for bird associated genospecies, suggesting a dilution effect. This difference between my results and those of Takumi *et al.* might reflect a difference in host communities between the Netherlands and the urban park chosen for my study. For instance, large ungulates

such as red deer (*Cervus elaphus*), wild boar and fallow deer (*Dama dama*), which are present in the Netherlands, were absent in my study. Food supplementation *per se* did not have any effects on DIN for *B. garinii* and *B. valaisiana* and these results suggest, once again, that birds that could have been attracted by food supplementation might have dropped fed larvae outside of trapping grids. My results suggest that the role of wood mice in driving DON can outweigh their potential role as dilution hosts for bird associated genospecies. However, because birds were not surveyed and could have been attracted by food, it is difficult to generalize the effects observed.

Using such experiments to manipulate host density through a change in resources and investigate the cascading effects on vector populations, pathogen prevalence and disease hazard allow for a better understanding of the mechanisms involved. It can also raise further questions about tick-host-pathogen interactions, guiding the path for future studies. Even though I used an experimental design here, the results suggest that various mechanisms are likely to be involved and must be investigated further. The dichotomy in this study lies in understanding whether food supplementation leads to an increase in ticks successfully feeding, as more hosts are available, or a decrease in tick successfully feeding because individuals are in better body condition and can fight off ectoparasites and pathogens, and both mechanisms are likely to happen simultaneously. One way to disentangle these effects would be to conduct an experiment in which the density of mice is controlled (e.g. using a mouse colony) and having some groups in good body conditions by providing nutritional food while other groups would be kept in poorer body conditions. A known number of tick larvae would feed on these individuals and looking at the proportion successfully feeding and moulting could help understanding which mechanisms are involved.

4.6. Study limitations

Recapture data showed that 44 mice moved between grids. While these individuals were removed from the analyses, I cannot be sure that the other mice strictly remained within one grid. Knowing how wood mice, with limited dispersal capacities, moved between grids, it is likely that other hosts (birds, squirrels, roe deer *Capreolus capreolus*) could have been attracted by food, picked up ticks in

supplemented grids and dropped them in surrounding woodlands, thus adding noise that could have obscured the predicted effects. Future studies need to be aware that it is likely to be important to also collect data on other hosts, especially birds and bank voles. In addition, I was expecting to see an impact of food supplementation *per se* on Lyme disease hazard, which was not the case here and I believe that this further suggests that other hosts were attracted by food supplementation but moved outside of trapping grids.

While I showed how food supplementation can increase wood mouse abundance, it would have been interesting to investigate the effects on body condition but I did not have the data. Similarly, I did not know which individual had received anti-helminthic treatment. While several studies showed that that similar treatment did not affect effect on Ixodid tick burdens, they focussed on ticks feeding on humans (Sheele *et al.*, 2013, 2014) and cattle (Taylor & Kenny, 1990) so the effects could be difference on wood mice and ticks attached to treated individuals could drop off and die, which would have affected tick burden data.

It was surprising that I did not detect *B. afzelii* in questing ticks, and future studies could investigate the reasons for the absence of *B. afzelii*. For instance, *B. afzelii* may go temporarily extinct (Mannelli *et al.*, 2012) and there could be a lag between an increase in small mammal population and the return of *B. afzelii*. The next step would be to test engorged ticks obtained from wood mice to see whether *B. afzelii* circulates in this system. In order to better understand the effects of tick burden on mice on DON, NIP and DIN, the next steps would be to identify life-stages of ticks removed from mice as well as identifying them at species level to look at the proportion of *I. ricinus* ticks feeding on mice compared to other tick species.

4.7. Conclusion

This experiment showed how fluctuations in resources directly affected wood mouse abundance. As expected, wood mouse abundance was positively correlated with DON. While *B. afzelii* was not detected in questing nymphs, I found an intriguing, nonlinear relationship between wood mouse abundance and DIN for *B. garinii* and *B. valaisiana*, highlighting the importance of wood mice in the Lyme

disease epidemiological cycle (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2018b; Takumi *et al.*, 2019). These results, along with the lack of effect from food supplementation *per se* on DON, NIP and DIN also raise questions about tick-host-pathogen interaction and warrants further studies, focussing on the wider host community and not just wood mice. More broadly, this experiment showed how pulsed availability of resources can strongly impact consumers' populations and how it can have repercussions on vector populations, pathogen prevalence and disease hazard and could apply to various pathogens as long as the consumer species is a competent reservoir host.

5. General discussion

5.1. Summary of findings

In this thesis, I investigated how host community composition affected Lyme disease hazard in Scottish woodlands. To understand the different relationships in place, it was essential to consider the entire ecological system, including interspecific interactions, environmental factors and anthropogenic pressure that might affect host species. In order to better understand this complex system, depending on both tick and host ecology as well as what affects them, I used two different approaches. In Chapter 2, I conducted a large cross-sectional survey at 28 sites to identify drivers for Lyme disease in Scotland while in chapters 3 and 4, I used experimental designs to focus on the specific roles that deer (Chapter 3) and wood mice (Chapter 4) play in the epidemiological cycle.

To summarize this work, my results show that Lyme disease hazard seems to be driven by the density of deer in the environment while the abundance of small mammals did not seem to matter, at least for *B. afzelii*. One would expect that the highest risk for Lyme disease would be in an environment supporting high densities of both deer and small mammals. However, this combination is unlikely to occur due to the negative effect of deer browsing pressure on small mammal communities. To my knowledge, this work is the first empirical study conducted in Europe to look at the role of both deer and small mammals simultaneously and to investigate the cascading effect of high deer density on Lyme disease dynamics through its effects on vegetation and small mammal communities.

These studies also highlight the importance to consider each genospecies as a unique pathogen. Indeed, for both the cross-sectional survey (Chapter 2) and the deer fencing experiment (Chapter 3), I observed that high deer densities led to higher risk for Lyme disease for *B. afzelii*. These results imply that the role of deer in driving tick populations overweighs the dilution effect observed (Chapter 2). While the deer fencing experiment (Chapter 3) only allowed me to test the effects of high deer density compared to the absence of deer, the cross-sectional (Chapter 2) survey offered the chance to show how DIN varied along the full range of deer density. However, the prevalence of *B. burgdorfer*i s.l. did not vary with

deer density, which could be due to the low prevalence of *B. burgdorfer*i s.l. in the study area but it could also be that different competent reservoir hosts (birds, squirrels) might be driving their associated genospecies in different ways.

While some studies showed that the effects of deer density on DON and thus DIN, plateaued at high densities (Kilpatrick *et al.*, 2017; Ostfeld *et al.*, 2006), I did not observe that in the cross-sectional study (Chapter 2), where DON and DIN seemed to increase linearly with deer density, even at high densities (>25 deer/km^{-2}). Hofmeester *et al.*, (2017) suggested that a linear relationship between deer density and DON may happen in ecosystems where deer are important hosts for immature ticks. In terms of management, this would imply that any reduction in deer density in Scotland could reduce Lyme disease hazard in the environment through their impacts on tick density.

To summarize my research findings I produced a diagram of two virtual ecosystems, showing the different factors influencing Lyme disease dynamics in Scottish woodlands (Figure 5.1). The two ecosystems created here reflect the findings from my studies.

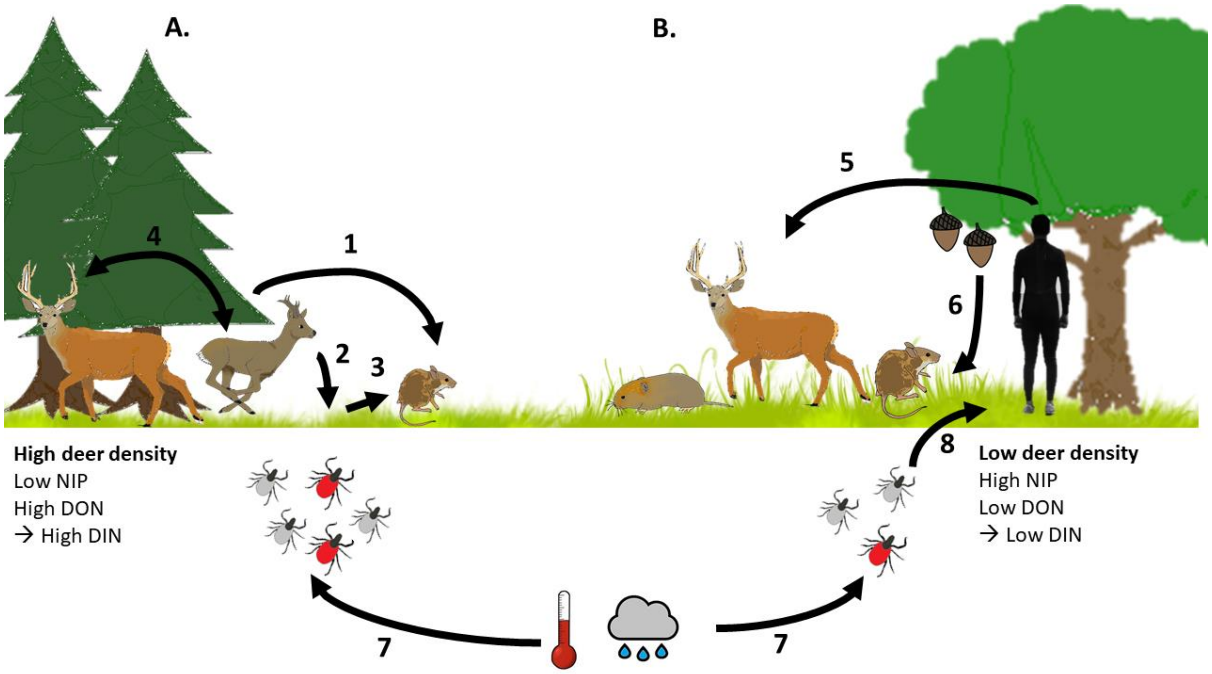


Figure 5.1: Conceptual figure illustrating the factors influencing host community composition and how the abundance of competent reservoir hosts (small mammals) and non-competent hosts (deer) and environmental factors affects the density of nymphs (DON), nymphal infection prevalence (NIP) and the density of infected nymphs (DIN). In system A, low anthropogenic pressure results in high densities of deer and the abundance of roe and red deer is dependent due to interspecific competition (4). This high density of reproduction hosts would result in fewer competent small mammals due to (1) direct disturbance and (2 & 3) shorter vegetation caused by grazing. With suitable environmental conditions (7) for off-host development, this host community would be predicted to result in a high DON and a low NIP, leading to a high DIN. In system B, (5) higher anthropogenic pressure would lead to lower densities of deer. Due to less browsing pressure, vegetation would be higher and denser and a higher small mammal density would be predicted. Small mammal density would also depend on (6) suitable resources in the environment. This host community would result in a lower DON and higher NIP, all of this leading to a lower DIN compared to system A. Contact rate between infected ticks and humans (8) would depend on DIN but also on human behaviour as some activities are likely to increase encounter rates between humans and ticks (e.g. hiking, mushroom picking etc.).

5.1.1. The direct role of deer in the Lyme disease cycle

When I designed the different studies and laid down my hypotheses, I expected deer to have a dual role in the Lyme disease epidemiological system due to their role as tick reproduction (Gilbert *et al.*, 2012; Mysterud *et al.*, 2016; Pacilly *et al.*, 2014) and dilution hosts (Huang *et al.*, 2018; Rosef *et al.*, 2009; Vourc'h *et al.*, 2016). As predicted, I observed a positive correlation between deer density and DON (Chapters 2 and 3), confirming their roles in driving vector populations. To further confirm their role as tick reproduction hosts, I surveyed for ticks in two

large fenced exclosures (Chapter 2) and I did not collect any questing ticks, which showed what to expect in a system lacking reproduction hosts (Gilbert *et al.*, 2012; Hofmeester *et al.*, 2017b). However, I did collect questing ticks in fenced exclosures in the deer fencing experiment (Chapter 3) and I believe that this was due to bank voles moving between plots, picking up ticks in high deer density plots and dropping them in deer exclusion plots.

Consistent with my predictions, I found evidence supporting the dilution effect hypothesis (LoGiudice *et al.*, 2003; Norman *et al.*, 1999; Ostfeld & Keesing, 2000c, 2000a; Schmidt & Ostfeld, 2001) in the cross-sectional survey (Chapter 2). Indeed, I observed a negative correlation between NIP for *B. burgdorferi* s.l. and *B. afzelii* and roe deer density. However, there was no association with red deer density, which may be due to differences between roe and red deer ecology (e.g. for differences in feeding ecology and home ranges: Kamler *et al.*, 2008; Kleveland, 2007; Latham *et al.*, 1999; Mysterud, 1999; Storms *et al.*, 2008; Vincent *et al.*, 1995) and these findings highlight the importance of considering roe and red deer as separate host as they could have different implications in the Lyme disease epidemiological cycle. In the deer fencing experiment (Chapter 3), I also observed a lower proportion of questing nymphs infected with *B. burgdorferi* s.l. in high deer density plots. Although this difference was not statistically significant, these results suggest that red deer could also play a role in diluting *B. burgdorferi* s.l.

To conclude on the direct role of deer on Lyme disease hazard, I found that high red deer density was associated with a 5-fold increase in Lyme disease hazard (deer fencing experiment, Chapter 3) (Takumi *et al.*, 2019; Vourc'h *et al.*, 2016), implicating that their role as reproduction hosts outweighed their role as dilution hosts. Regarding the results from the cross-sectional survey (Chapter 2) there was no association between DIN with *B. burgdorferi* s.l. and deer density (both roe and red deer). However, DIN for *B. afzelii* was positively correlated with both roe and red deer density. It must be noted that 92% of ticks infected with *B. burgdorferi* s.l. in the deer fencing experiment (Chapter 3) were infected with *B. afzelii,* so it is possible that I would have observed a similar lack of association between deer and the density of infected nymphs for *B. burgdorferi* s.l. if more questing ticks had been infected with other genospecies. I would expect to observe a similar pattern for all genospecies and the difference in the association

between deer density and DIN between *B. burgdorferi* s.l. and *B. afzelii* observed in the cross-sectional survey might be due to the low infection rate in the cross-sectional study. Indeed, the NIP was 2% (95%CI: 1.8-2.2), which did not provide the models with much variation and few ticks were infected with genospecies other than *B. afzelii* (NIP for *B. afzelii*: 60%, n=177, for *B. garinii:* 16%, n=48, for *B. valaisiana* and *B. burgdorferi* s.s.*:* 9%, n=26).

5.1.2. The direct role of small mammals in the Lyme disease cycle

Small mammals, which are competent reservoirs for *B. afzelii*, are important components in the Lyme disease epidemiological cycle as they provide blood meals for immature ticks and can drive *B. afzelii* prevalence (Mysterud *et al.*, 2016; Ostfeld *et al.*, 2001; Takumi *et al.*, 2019; Vuong *et al.*, 2017). In the cross-sectional survey (Chapter 2), the trapping data allowed me to confirm that wood mice harboured, on average, more individual ticks compared to bank voles (Perez *et al.*, 2016; Tälleklint & Jaenson, 1997; van Duijvendijk, 2016). The prevalence of *B. afzelii* in questing ticks was also higher in September compared to May and July (Coipan *et al.*, 2013; Reye *et al.*, 2010). This could suggest that small mammal abundance increases along the summer and the higher NIP I observed in September might indicate larvae that fed on small mammals in the spring and moulted in the same season.

In the wood mouse experiment study (Chapter 4), wood mouse abundance was increased using food supplementation and I investigated the effects on tick burden on wood mice, DON, NIP and DIN. Consistent with my predictions, food supplementation resulted in an increase in wood mouse abundance the same year, as seen in other studies (Krawczyk *et al.*, 2020; van Duijvendijk, 2016). Food supplementation *per se* had no effect on tick burden on wood mice the same year, nor on DON, NIP and DIN the spring following food supplementation. Although I predicted that wood mouse abundance should result in lower tick burdens on wood mice, there was no evidence to support this hypothesis in my results. I also expected wood mouse abundance to affect NIP the following spring (the direction of this effect depending on several hypotheses) but there was no association between wood mouse abundance and NIP for *B. garinii* and *B. valaisiana* (no ticks were infected with *B. afzelii*). Consistent with my first hypothesis, DON the

following spring was positively correlated with wood mouse abundance, which could be a result of more larvae successfully feeding on wood mice the previous year (Krawczyk *et al.*, 2020; Ostfeld *et al.*, 2006). There was also a positive association between wood mouse abundance and DIN for *B. garinii* and *B. valaisiana*, suggesting that wood mice might play a role in driving Lyme disease hazard via their positive effects on tick density.

5.1.3. The combined role of deer and small mammals in the Lyme disease cycle

While investigating the roles of deer and small mammals independently on Lyme disease transmission risk helped disentangled some mechanisms involved in this complex system, the novelty of this work was to investigate their parts when together. Even though the deer fencing experiment (Chapter 3) focussed on the effects of deer on Lyme disease hazard, I was able to also study bank voles and I showed that small mammal communities can be strongly affected by deer. Indeed, high deer density resulted in a strong decline in bank vole abundance, likely through a change in vegetation. I concluded that even if an ecosystem harbours a high abundance of competent reservoir hosts, it will lead to higher risk of Lyme disease only if there are enough ticks in the environment for pathogen transmission (Logiudice *et al.*, 2008) and in this case, I showed that Lyme disease hazard was higher in high deer density plots even though small mammal abundance was low. Earlier results from the cross-sectional survey (Chapter 2) confirmed this. Indeed, when I considered small mammals and deer together, there was a decline in NIP for *B. afzelii* as a function of roe deer density (Rosef *et al.*, 2009; Vourc'h *et al.*, 2016), consistent with a dilution effect in sites with both low and high small mammal abundance. This could imply that high deer density might mitigate disease hazard through a dilution effect. However, I found a positive correlation between both roe and red deer density and DIN for *B. afzelii* (Takumi *et al.*, 2019; Vourc'h *et al.*, 2016), confirming that Lyme disease hazard might be driven by deer density rather than small mammals, within the combination of host densities in this study. I was expecting to see a stronger effect of small mammal abundance on both NIP and DIN for *B. afzelii* and I hypothesised that this inability to detect such effect might have come from the fact that only a few sites had high abundance of small mammals.

5.1.4. The drivers of host community composition

While the central aim of this thesis was to investigate the effects of host community composition on Lyme disease transmission risk, I realized that, if Lyme disease hazard is dependent on the density of deer and small mammals, I had to take into account the factors influencing the density of these hosts to get a full picture.

Regarding deer, I observed a negative association between roe and red deer, which could suggest interspecific competition (Latham *et al.*, 1996; Latham & Staines, 1997) and roe deer density was negatively associated with trail density (Bonnot *et al.*, 2013; Hewison *et al.*, 2001; Scholten *et al.*, 2018), a proxy for human activity while the same was not true for red deer (cross-sectional survey, Chapter 2). This is very interesting as it suggests that anthropogenic pressure might affect deer distribution and have repercussions on Lyme disease hazard.

Regarding small mammals, food supplementation led to an increase in wood mouse abundance, which had a positive impact on Lyme disease hazard (Chapter 4) (Krawczyk *et al.*, 2020; Rubel & Brugger, 2020; van Duijvendijk, 2016). Variations in resources are common occurrences in nature and could replicate conditions similar to those of this experiment. In the context of climate change, a study has shown how increasing temperatures could result in a rise in the frequency of mast seeding events (Touzot *et al.*, 2020), which could thus affect disease dynamics (Ostfeld *et al.*, 2001; Rubel & Brugger, 2020; van Duijvendijk, 2016).

I established how deer can affect Lyme disease transmission risk by driving tick populations but also by reducing the proportion of infected ticks. During this work, I also brought evidence for their third potential role in mitigating or amplifying disease hazard in their capacity of shaping ecosystems. Indeed, high browsing pressure led to shorter and sparser vegetation (Buesching *et al.*, 2011) and, besides the consequences that this could have on forest regeneration, I also demonstrated that this negatively affected small mammal communities (Chapter 3) (Flowerdew & Ellwood, 2001; Smit *et al.*, 2011; van Wieren & Bakker, 2008). High deer density led to a 13-fold decrease in bank vole abundance and this was further confirmed in the cross-sectional survey (Chapter 2) where I observed a

negative correlation between deer density and wood mouse abundance. This could have implications for other diseases as small mammals are competent reservoir hosts for many pathogens (Holmberg *et al.*, 2003; Mills *et al.*, 1999; Reed *et al.*, 2003; Riehm *et al.*, 2011) and high densities of deer could lower disease hazard by reducing competent reservoir host density.

5.2. Study limitations

In Chapter 2, I surveyed 28 sites across Aberdeenshire to sample ecosystems with a range of host density that would be representative of Scotland. However, due to time and resource constraints, it was not always possible to collect the optimal type and/or amount of data. First, I used a proxy to estimate deer density (deer dung count) (Acevedo *et al.*, 2010; Mayle *et al.*, 1999), which I found to correlate well with actual deer density and should thus be accurate. One caveat for this study though, is that deer density for a site did not necessarily reflect how deer were using their habitat. Indeed, counting 100 pellets could be due to either a herd of 50 deer passing through the site quickly and dropping pellets or a few deer spending a long time in the same area, which would not have the same consequence for ticks. Indeed, if a herd of deer passed through the site when climatic conditions were not adequate for tick questing (e.g. freezing temperatures, dry day), no ticks would have been able to feed on these deer while a few deer staying in the area would allow ticks to feed and drop off in the vicinity. I believe that combining deer dung count with other methods such as GPS tracking of tagged individuals and/or camera trapping might provide more information on habitat use.

Thinking back to *Ixodes ricinus* life cycle (see Figure 1.9), there should be a one to two-year lag between deer density and questing nymph abundance. Questing ticks collected at $year_t$ would have hatched into larvae at $year_{t-1}$ that may come from adult females which fed on deer at $year_{t-2}$. I logistically could not wait two years after deer density estimation before collecting questing nymphs. However, future studies should, if possible, take this time-lag into account when collecting data on host abundance and questing ticks.

Regarding small mammal trapping, I had to make a trade-off between trapping for a long period of time in a few sites or trapping fewer nights in more sites. I decided on the latter and I only trapped for two consecutive nights in each site. Sampling for longer would also have brought the issue of small mammals reproducing and sites surveyed early in the season may not have been comparable to those surveyed later in the summer. While the method I chose provided comparable abundance of small mammals across the sites, it would have been beneficial to trap for longer. That would have allowed me to increase my sample size and I might have been able to separate bank voles and wood mice in the analyses as they are likely to play different role in the Lyme disease epidemiological cycle (Perez *et al.*, 2016). In this study, I wanted to work with a simplified host community so I deliberately chose sites that did not have sheep and I conducted the study in areas where grey squirrels are absent. In addition, I could not trap shrews, which are competent reservoir hosts (Brisson & Dykhuizen, 2004; LoGiudice *et al.*, 2003), as they are protected species and necessitate a special trapping license. However, I am aware how important these hosts can be in some cases (Millins *et al.*, 2015; Ogden *et al.*, 1997; Salkeld *et al.*, 2008). Lastly, I was unable to record bird density due to logistic reasons and it would have been interesting to investigate the effects of the ratio of birds and deer on Lyme disease hazard, focussing on *B. garinii* and *B. valaisiana* as this has not been done before.

In the deer fencing experiment (Chapter 3), I decided to use an experimental design controlling deer densities to assess more clearly the role of deer in the Lyme disease transmission cycle. I had extensive data on vegetation and tick abundance (4 years of data) and I knew exactly how many deer were kept in the enclosure. In contrast, the data collection for small mammals could have been improved. I trapped small mammals for two years and the lack of trapping success in high deer density plots meant that I could not compare individual tick burden between treatments (high deer density vs absence of deer), which would have been useful. To better understand how small mammals moved within and between plots, it would have been interesting to track their movements. The plots I used were small (0.25 Ha) and one study has criticised the use of such small enclosures (Perkins *et al.*, 2006) because they found that it led to tick amplification, even 16 years after implementation. However, despite the small scale I found a strong effect of deer exclusion on DON, NIP and DIN. From these results, I can infer that

the effect would have been even stronger at a larger scale (Gilbert *et al.*, 2012; Hofmeester *et al.*, 2017b). This was confirmed in the cross-sectional survey, as I did not collect any ticks in the two large scale exclosures I used (site GLS: 5.11 Ha, site GSB: 1 Ha). The density of deer in the deer fencing experiment was high (32.47 red deer/km^{-2}) and might not reflect those in natural woodlands. However, four sites (out of 28) in the cross-sectional study were estimated to have similar densities (BSG: 32.58 deer/km^{-2}, GT1: 31.59 deer/km^{-2}, SLE: 32.08 deer/km^{-2} and WOOD: 30.85 deer/km^{-2}).

In Chapter 4, I used an experimental design to investigate the effects of an increase in wood mouse abundance using food supplementation on Lyme disease hazard. Small mammal densities are known to fluctuate (Lambin *et al.*, 2000; O'Mahony *et al.*, 1999; Redpath *et al.*, 2002), mainly due to predation (Hanski *et al.*, 2001; Korpimäki *et al.*, 2002; Nie & Liu, 2005) and variations in resource availability (Clotfelter *et al.*, 2007; McShea, 2000; Ostfeld *et al.*, 2006). Because it was logistically impossible for me to conduct extensive small mammal trapping in this site as I was already doing field work for the other two studies, I took the opportunity offered by Dr. Amy Pedersen and her group from the University of Edinburgh and used their extensive data set on wood mouse trapping. While their study design was robust for the purpose of their study, I found that some modifications to the design would have made it better for my study. For example, this includes having grids more spaced out to reduce the possibility of wood mouse movement between grids and collecting data on additional hosts that would have benefited from food supplementation and could have influenced tick populations and pathogen transmission (e.g. birds, squirrels, bank voles).

While the three studies conducted for this thesis should be applicable to any part of the United Kingdom, they might not be transferable to mainland Europe and North America where the host community is richer (Baquero & Tellería, 2001; Kier *et al.*, 2009). Even though these studies were conducted over several years, I only looked at a small window that does not give me information on what had happened to these sites prior these studies or what would happen if there was a change in host community composition.

Lastly, the change of method used to detect *B. burgdorferi* s.l. halfway through this thesis might have impacted my results. Before making this decision, I extensively tested for differences in sensitivity and specificity with both methods but found none, and I also decided to remove positive samples that could not be sequenced from analyses focussing on specific genospecies. When applicable, I also included the PCR method as a fixed term in my statistical models.

5.3. Future work

When investigating the role of deer in the cross-sectional survey (Chapter2), the most important aspect for future work would be to look at deer habitat usage using, for example, a mix of GPS data, aerial footage and/or camera trapping to identify hotspots where deer spend their time and understand how they use their habitat. A gradient of deer usage could then be surveyed for ticks and small mammals to have a more accurate representation of their role in the Lyme disease epidemiological cycle. Furthermore, the impacts of anthropogenic pressure (density of hiking trails) should be investigated further as it could be crucial for Lyme disease dynamics. Indeed, high human activities could lead to fewer deer, resulting in more small mammals and potentially higher prevalence if enough ticks are present in the environment. A long-term empirical study recording the entire host community, including shrews, birds and squirrels, would also bring precious data but it would be logistically difficult to implement.

As mentioned in the caveats, if someone wanted to build on the work conducted in the deer fencing experiment (Chapter 3), the next step would be to track bank voles and see how they moved through the landscape. To test whether lower bank vole abundance in deer exclusion plots is a result of direct disturbance from deer or due to loss of cover, it would be interesting to create a third treatment by having plots fenced against deer but keep the vegetation short. If the abundance of bank vole in this third treatment is higher than in high deer density plots, it would suggest that bank voles are directly impacted by deer trampling while, if the abundance is similar to that of high deer density plots, it would imply that bank voles are affected by the short vegetation.

Regarding Chapter 4, the next steps would be to test engorged ticks obtained from wood mice to see whether *B. afzelii* circulates in this system. It would also be interesting to identify tick species feeding on wood mice as only *I. ricinus* nymphs were collected from the vegetation whereas other species could feed on rodents. A study conducted in Scotland found that 92% of larvae collected on rodents were *I. ricinus* and 4% were *I. trianguliceps* (James, 2010) while in Norway, 73% of larvae on wood mice were *I. ricinus* and 23% were *I. trianguliceps* (Mysterud *et al.*, 2015). In terms of modelling, it would be interesting to make predictions based on mast seeding events as resource fluctuations might become more frequent with climate change (Touzot *et al.*, 2020) and could lead to a temporal increase in competent hosts in the environment, thus affecting disease dynamics.

5.4. Implications for management

In terms of management, my results suggest that removing deer from a system would likely result in a strong decrease in Lyme disease hazard once the system has stabilized as no vectors should remain (Gilbert *et al.*, 2012; Hofmeester *et al.*, 2017b). Indeed, tick density might first increase following deer exclusion as there are no longer deer to feed on and more ticks are questing for hosts (Perkins *et al.*, 2006; Rand *et al.*, 2004). However, it would be ethically and logistically challenging to completely remove deer from large areas by culling. The use of deer fences could be used as a management tool, providing that they allow other species to circulate and, based on my results from the cross-sectional survey, no ticks should be found in exclosures. Fencing large areas against deer might present some challenges as it would be expensive and would require maintenance. Based on the results from the cross-sectional study (chapter 2), any reduction in roe and red deer density is likely to reduce the number of ticks in the environment and the density of nymphs infected with *B. afzelii*, which is the dominant genospecies in Scotland (James *et al.*, 2013, 2014; Millins *et al.*, 2016) and this could be implemented more easily by increasing hunting pressure. Because the management of deer might present several issues (e.g. expensive to implement, difficulty to remove deer from large areas), other means of reducing contact rates between humans and ticks are needed.

Keeping awareness campaigns and flyers in woodlands where human activity is high could be a useful tool to make the public aware of a potential risk in certain areas and explaining how to reduce exposure risk by checking for tick bites after any outdoor activity and using repellent. In terms of management, cutting the vegetation along footpaths may reduce contact rates between humans and ticks. In a similar manner, creating wider paths could prevent tick bites as humans would not be in contact with the vegetation.

5.5. Applicability to other systems

The effect of host community on other tick-borne pathogens has not been extensively studied (Tick-borne encephalitis: Cagnacci *et al.*, 2012, anaplasmosis: Estrada-Peña *et al.*, 2008). However, it is important to take into consideration the combined effect of both competent reservoir hosts and non-competent hosts as they could affect each other. In regard to tick-borne diseases, the results from this work should be applicable to other pathogens as long at the vector species is a generalist and requires specific reproduction hosts that are non-competent hosts. For other vector-borne diseases though, it might not be relevant due to differences in vector ecology. For example, mosquito development depends on environmental conditions (Roiz *et al.*, 2014; Valdez *et al.*, 2017) and female mosquitoes only feed for a few seconds so a range of range of hosts would be suitable for females to reproduce. Ticks have a very particular life cycle and are the only vectors that need three different hosts to complete their life cycle and must stay attached to their hosts for several days to complete their blood meals. If intense browsing pressure by large ungulates has a negative impact on small mammal abundance, other pathogens carried by small mammals (e.g. hantavirus, *Yersinia pestis*, Lassa virus, *Leptospira* complex) might be affected and disease hazard could vary and, to my knowledge, no study has investigated this. Although other studies have looked at disease mitigation through ecological cascades (Hofmeester *et al.*, 2017a; Hoyer *et al.*, 2017; Levi *et al.*, 2012), these are few and focussed on Lyme disease and it would be interesting to see if it applies to other systems.

Using field experiments in disease ecology present many challenges compared to controlled experiments in a laboratory environment. Indeed, there are many

factors in natural ecosystems that can affect vectors and hosts (e.g. climatic conditions, interspecific interactions) and cannot be accounted for and/or controlled, resulting in noise that can make it difficult to separate the relationships of interest. While lab experiments allow to control for all these different factors and separate processes, results might not necessarily apply in natural environments because of the role these other factors play, which could affect mechanisms and relationships. In regard to Lyme disease, I believe that a mixture between laboratory and field experiments is needed to decorticate the different mechanisms in place and understand what drives Lyme disease in natural ecosystems.

To conclude on this four year journey, and if I had to summarize my thesis in one sentence, I would say that ticks are truly fascinating creatures, that small mammal abundance is not that important in the Lyme disease epidemiological cycle compared to deer, which really are the ones driving disease hazard in Scottish woodlands.

Appendix A: Supplementary materials for Chapter 2

S1: Characteristics of field sites

Table A-S1.1:Table summarizing the habitat type (Conif.: coniferous, Deci.: deciduous, mix.: mixed forests), mean roe deer density/km^{-2}, mean red deer density/km^{-2}, mean small mammal index/10m, mean small mammal abundance/100 trap nights, mean density of questing nymphs/10m (DON) and number of nymphs tested for *B. burgdorferi* s.l. prevalence for each of the 28 sites surveyed. Site highlighted in grey were only samples one year due to forestry work and sites highlighted in orange were the two exclosures used for which no ticks could be tested.

Site	Habitat	Roe deer	Red deer	Small mammal index	Small mammal abundance	DON	No. tested
BALO	Conif.	13.1	6.3	1.0	0	2.4	601
BER	Conif.	6.0	2.4	0.8	0.5	2.5	698
BGA	Conif.	11.1	9.0	0.2	0	5.8	500
BOD	Conif.	16.1	12.6	0.9	0	2.0	296
BOG	Conif.	14.1	1.6	5.7	4.5	3.5	598
BSG	Conif.	15.1	6.3	0.7	0.5	3.6	599
COM	Conif.	2.0	0	0.2	0.9	0.6	596
DNR	Deci.	8.0	0	0	1.2	3.5	597
FORD	Conif.	8.0	7.9	0.2	1.0	5.3	500
GB	Deci.	1.0	0	1.3	0	0.9	600
GEN	Conif.	1.0	0	0	1.0	1.1	597
GLS	Conif.	0	0	2.0	0	0	0
GSB	Conif.	0	0	2.3	3.3	0	0
GT1	Conif.	11.1	20.5	0.2	1.0	9.	498
HP	Conif.	9.0	0	3.7	0	1.1	481
KNW	Conif.	1.0	0	0.8	0.5	0.6	298
MOC	Conif.	9.0	1.6	0.1	3.5	1.7	599
MOD	Mix.	15.1	4.7	0.5	0	3.6	300
MODNE	Deci.	1.0	0.8	0.1	2.0	1.0	591
MODNW	Mix.	11.1	7.9	0.2	1.6	2.3	596
MODSW	Mix.	10.0	6.3	0.2	1.4	2.0	589
MT	Conif.	4.0	0	0.5	0	0.8	485
MUN	Conif.	15.1	0	0.8	9.6	2.2	500
NSFF	Mix.	14.1	1.6	0.1	5.0	1.9	492
SCF	Conif.	2.0	0	0.8	2.8	0.9	593
SFHW	Conif.	12.1	1.6	0.2	1.3	0.6	534
SLE	Conif.	2.0	24.5	1.2	0	6.8	500
WOOD	Conif.	26.1	4.7	0.3	0.5	13.8	500

S2: Molecular methods used to test questing nymphs

Table A-S2.1: Prevalence of *Borrelia burgdorferi* sensu lato (%) and number of ticks tested for each site in May, July and September for two consecutive years. Shaded cells indicate the samples tested using the qPCR while the rest indicate samples tested using the nested PCR method.

Site	2017			2018		
	May	July	September	May	July	September
NSFF	1 (100)	0 (93)	1.1 (92)	2.0 (99)	0 (100)	0 (100)
SCF	14 (100)	8 (100)	13.3 (98)	3.1 (96)	7.0 (100)	5.1 (99)
SFHW	0 (42)	0 (100)	5.1 (99)	0 (100)	0 (93)	5 (100)
GB	1 (100)	2 (100)	4 (100)	6 (100)	2 (100)	1 (100)
KNW	2 (100)	1 (98)	1 (100)		Forestry work	
MOC	5 (100)	1 (100)	1 (99)	1 (100)	0 (100)	5 (100)
BOG	5 (101)	1 (100)	0 (100)	3 (100)	0 (97)	0 (100)
BALO	2 (100)	1 (100)	0 (100)	0 (100)	1 (101)	3 (100)
BER	1 (100)	0 (100)	0 (99)	0 (100)	1 (99)	6 (100)
BSG	4 (99)	1 (100)	0 (100)	4 (100)	1 (100)	0 (100)
DNR	1 (97)	0 (100)	1 (100)	1 (100)	1 (100)	0 (100)
COM	6 (100)	1 (100)	1 (98)	5 (100)	0 (98)	5 (100)
MOD	0 (100)	0 (100)	0 (100)	0 (100)	0 (99)	0 (100)
MODNW	0 (99)	0 (100)	0 (100)	2 (98)	0 (99)	2 (100)
MODSW	2.1 (95)	1 (100)	0 (100)	1 (100)	0 (95)	1 (100)
MODNE	1 (100)	3 (100)	0 (100)	0 (91)	0 (100)	0 (100)
BOD	0 (99)	0 (97)	0 (100)		Forestry work	
GLS			No ticks collected			
GSB			No ticks collected			

	2018			2019		
	May	July	September	May	July	September
FORD	2 (100)	2 (100)	0 (100)	2 (100)	8 (100)	4 (100)
SLE	4 (100)	5 (100)	2 (100)	2 (100)	5 (100)	3.6 (83)
WOOD	0 (100)	0 (100)	1 (100)	0 (100)	0 (100)	1 (100)
BGA	0 (100)	1 (100)	1 (100)	5 (100)	3 (100)	10 (98)
MT	0 (100)	2.2 (91)	2.1 (95)	1 (99)	5 (100)	3.5 (85)
MUN	2 (100)	0 (100)	6 (100)	1 (100)	1 (100)	2 (100)
HP	2 (100)	0 (93)	3.1 (98)	0 (98)	0 (92)	2 (99)
GT1	0 (100)	0 (100)	0 (100)	1 (98)	0 (100)	1 (100)
GEN	3 (100)	0 (97)	4 (100)	9 (100)	10 (100)	8.8 (80)

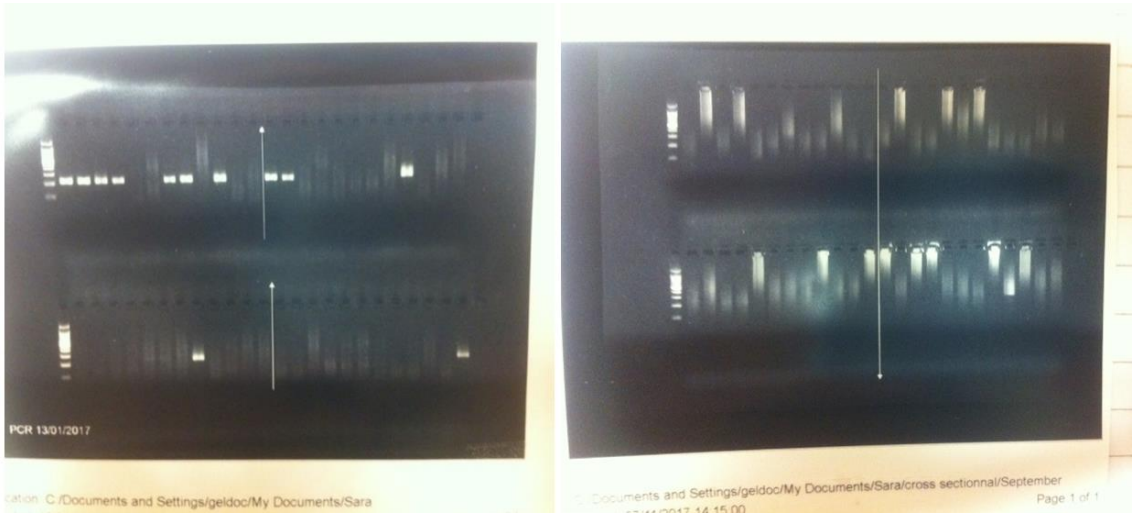


Figure A-S2.1: Images of gels obtained with the nested PCR protocol. The one on the left was done in 2017 and positive samples clearly appear on the gel with thin bands. The image on the right is from a nested PCR done in 2018 where smears make the interpretation of the gel difficult as they could hide positive samples. I decided to switch to a qPCR protocol after trying to resolve this issue for more than 12 months.

S3: Permutation tests to assess deer population stability

I used a permutation test to assess deer stability for each site between the two years of sampling. Permutation tests build sampling distribution by resampling the observed data. In this case, the null hypothesis was that the group label (i.e. the density of deer the first and second year for each site) was arbitrary and switching groups should not affect the outcome. Results from the permutation test can be found in Table 3 and it appeared that deer density was stable for all the study sites. I used a Bonferroni correction to reduce type I error.

Table A-S3.1: output from permutation tests used on the density of deer for each site during the first and second year of sampling. P-value adjusted represents the p-value from the test with a Bonferroni correction.

Site	p-value adjusted	Site	p-value adjusted
BALO	0.90	MOD	0.88
BER	0.34	MODNE	1.0
BGA	0.34	MODNW	1.0
BOG	0.22	MODSW	0.91
BSG	0.06	MT	0.48
COM	1.0	MUN	0.10
DNR	0.32	NSFF	0.15
FORD	0.47	SCF	0.66
GEN	0.40	SFHW	0.43
GT1	0.65	SLE	0.45
HP	0.05	WOOD	0.11
MOC	0.06		

Additionally, I used a Pearson's correlation test to assess the correlation in average deer density between the two years of collection and we found a strong correlation between the estimated density in the first year compared to the second year (r_p=0.78, p<0.001).

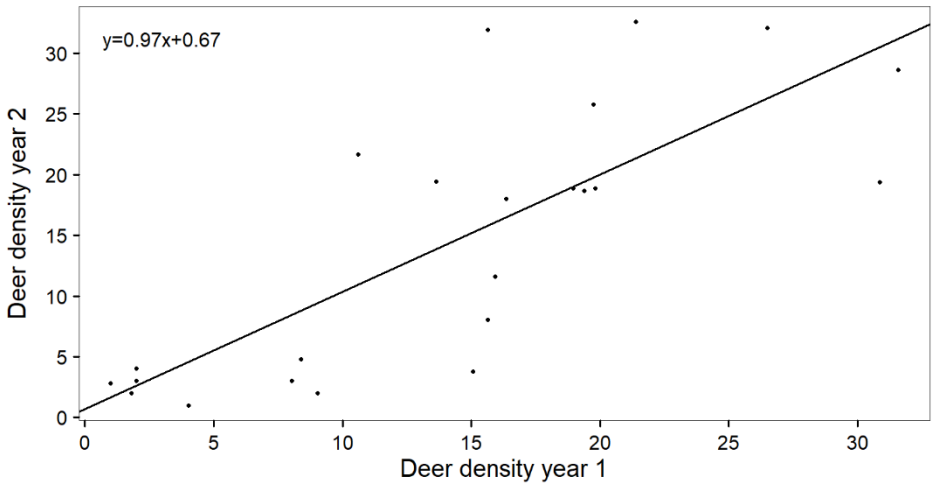


Figure A-S3.1: Scatterplot representing the density of deer during the first year of survey against the density during the second year of survey.

S4: Dividing small mammal abundance into a categorized index

Table A-S4.1: Table representing the 29 sites surveyed, the average number of individual wood mice and bank voles (no recapture) captured per 100 trap nights, the average number of vole signs/10m the same year as live trapping took place and how these were divided in categorical indexes.

Site	Small mammals /100TN	Small mammal index	No. of vole signs/10m	Vole sign index	Combined index
NSFF	5.0	High	0.1	Low	High
MOC	3.5	High	0.1	Low	High
SCF	2.8	High	0.8	Low	High
KNW	0.5	Low	0.8	Low	Low
BSG	0.5	Low	0.7	Low	Low
BOG	4.5	High	5.7	High	High
BER	0.5	Low	0.8	Low	Low
BALO	0	Low	1.0	High	High
DNR	1.2	Low	0	Low	Low
MOD	0	Low	0.5	Low	Low
MODNE	2.0	High	0.1	Low	High
SFHW	1.3	Low	0.2	Low	Low
MODNW	1.6	Low	0.2	Low	Low
COM	0.9	Low	0.2	Low	Low
MODSW	1.4	Low	0.2	Low	Low
BOD	0	Low	0.9	Low	Low
GB	0	Low	1.3	High	High
GSB	3.3	High	2.3	High	High
GLS	0	Low	2.0	High	High
FORD	1.0	Low	0.2	Low	Low
WOOD	0.5	Low	0.3	Low	Low
BGA	0	Low	0.2	Low	Low
MUN	9.6	High	0.8	Low	High
GEN	1.0	Low	0	Low	Low
SLE	0	Low	1.2	High	High
HP	0	Low	3.7	High	High
MT	0	Low	0.5	Low	Low
GT1	1.0	Low	0.2	Low	Low

S5: The effects of deer density on questing nymph abundance

Nymph abundance model focussing on roe deer density: full summary table

Table A-S5.1: Results from the selected model to explain what causes variations in questing nymph density for the model focussing on roe deer.

Variable	Estimate (log)	Std Error	z-value	p-value
Intercept	0.34	0.24	1.44	0.15
Temperature	-0.03	0.03	-1.24	0.22
Temperature^2	-0.08	0.01	-6.09	<0.001
Ground Vegetation: EV	-0.10	0.11	-0.95	0.34
Ground Vegetation: G	-0.40	0.11	-3.75	<0.001
Ground Vegetation: M	-0.73	0.15	-4.83	<0.001
Ground wet: yes	-0.96	0.09	-11.17	<0.001
Visit: May	0.21	0.06	3.69	0.00
Visit: September	0.55	0.05	10.46	<0.001
Habitat: Deciduous	0.78	0.66	1.18	0.24
Habitat: Mixed	-0.22	0.12	-1.95	0.05
Roe deer density year$_t$	-0.06	0.04	-1.67	0.09
Vegetation height	-0.12	0.03	-4.46	<0.001
Year: 2018	0.53	0.06	8.91	<0.001
Year: 2019	0.55	0.08	6.47	<0.001
Rainfall previous day	-0.11	0.02	-4.57	<0.001
Habitat: Deciduous*Roe deer	0.80	0.25	3.26	0.001
Habitat: Mixed*Roe deer	-0.06	0.10	-0.60	0.55

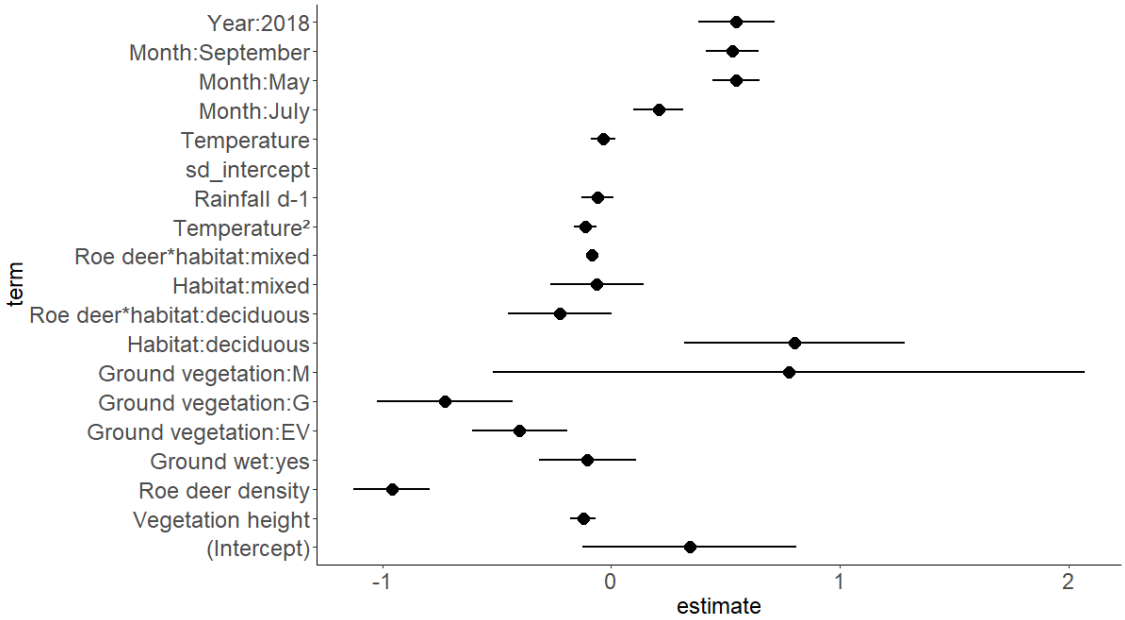


Figure A-S5.1: Effects of fixed variables on questing nymph abundance.

Table A-S5.2: Results from the Tukey HSD test representing the difference the average density of questing nymphs (fitted values) depending on year, ground vegetation and season.

	Estimate (log)	Std Error	z-value	p-value
2018-2017	0.53	0.06	8.91	<0.001
2019-2017	0.55	0.08	6.47	<0.001
2019-2018	0.02	0.06	0.31	0.94
EV-BF	-0.10	0.11	-0.95	0.76
G-BF	-0.40	0.11	-3.75	< 0.001
M-BF	-0.73	0.15	-4.83	< 0.001
G-EV	-0.30	0.06	-5.08	< 0.001
M-EV	-0.62	0.11	-5.45	< 0.001
M-G	-0.33	0.11	-3.12	0.01
May-July	0.21	0.06	3.69	< 0.001
September-July	0.55	0.05	10.46	< 0.001
September-May	0.34	0.05	6.43	< 0.001

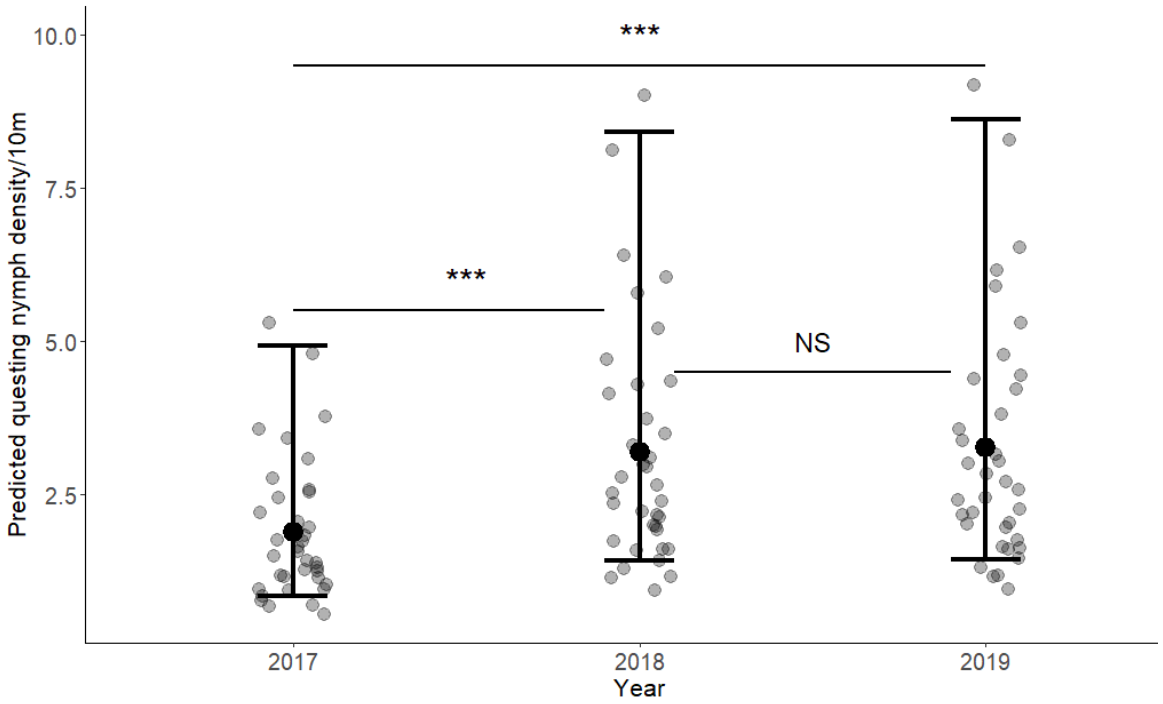


Figure A-S5.2: Predicted density of questing nymphs/10m in 2017, 2018 and 2019. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

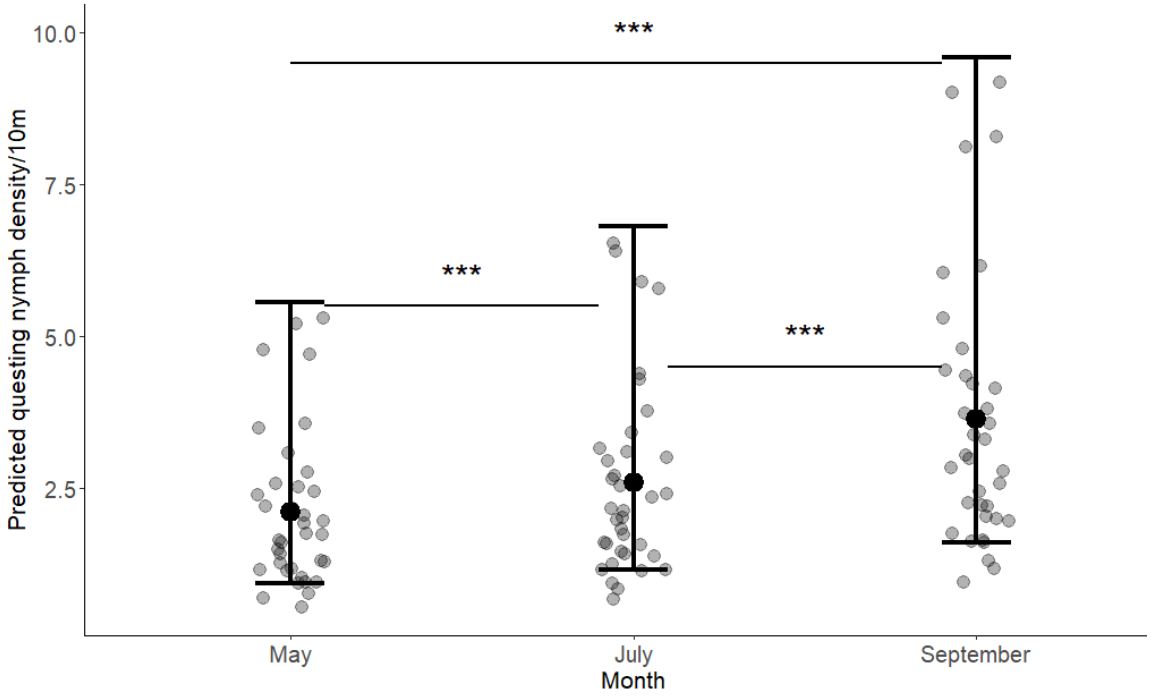


Figure A-S5.3: Predicted density of questing nymphs/10m in May, July and September. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

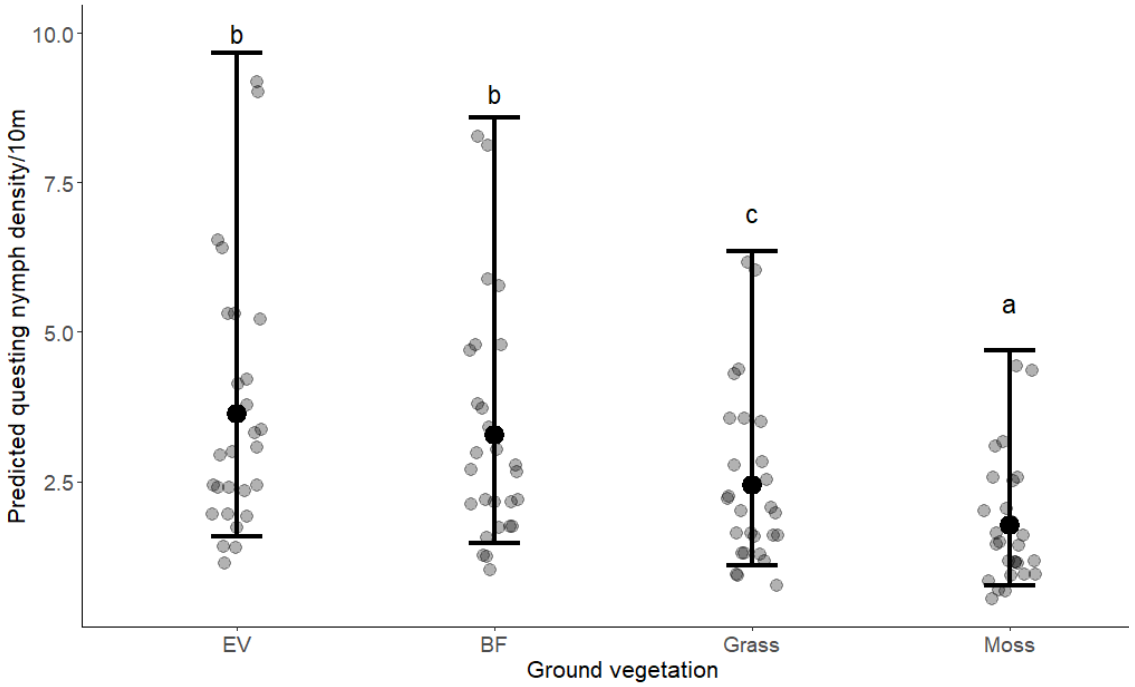


Figure A-S5.4: Predicted density of questing nymphs/10m depending on ground vegetation type. Different letters (a, b and c) show significant difference among groups based on Tukey HSD test.

Nymph abundance model focussing on red deer density: full summary table

Table A-S5.3: Results from the selected model to explain what causes variations in questing nymph density for the model focussing on red deer.

Variable	Estimate (log)	Std Error	z-value	p-value
Intercept	0.28	0.21	1.33	0.18
Temperature	-0.03	0.03	-1.10	0.27
Temperature^2	-0.07	0.01	-5.25	<0.001
Ground Vegetation: EV	-0.08	0.11	-0.75	0.45
Ground Vegetation: G	-0.38	0.11	-3.49	<0.001
Ground Vegetation: M	-0.68	0.16	-4.40	<0.001
Ground wet: yes	-0.97	0.09	-11.10	<0.001
Visit: May	0.22	0.06	3.82	<0.001
Visit: September	0.57	0.05	10.65	<0.001
Red deer density year$_t$	0.15	0.04	3.41	<0.001
Vegetation height	-0.12	0.03	-4.09	<0.001
Year: 2018	0.50	0.06	8.97	<0.001
Year: 2019	0.63	0.09	6.72	<0.001
Rainfall previous day	-0.12	0.03	-4.77	<0.001

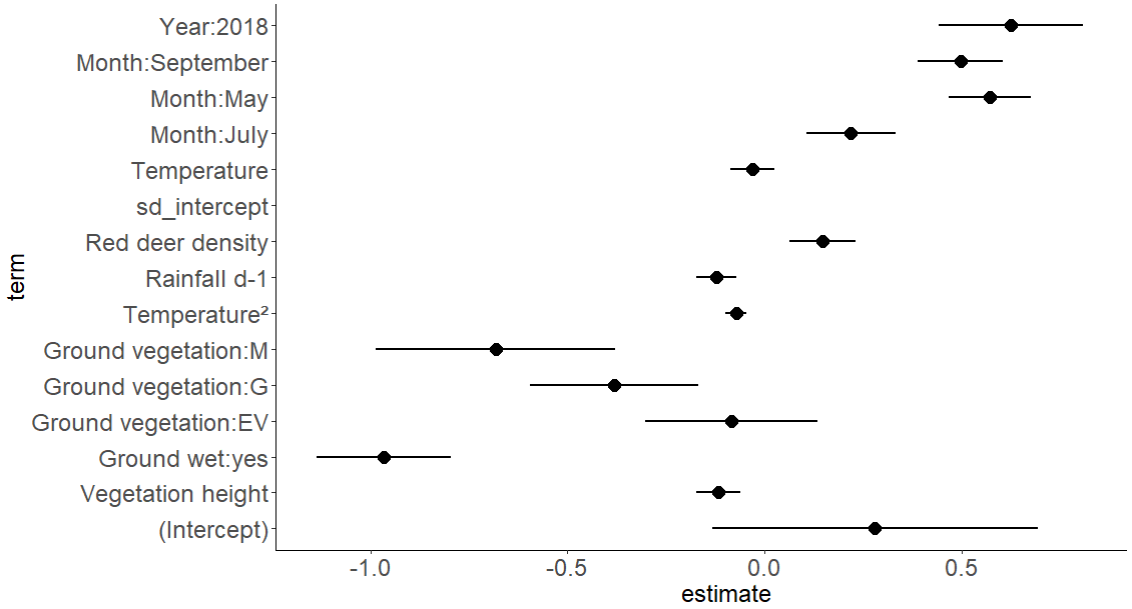


Figure A-S5.5: Effects of fixed variables on questing nymph abundance.

Table A-S5.4: Results from the Tukey HSD test representing the difference the average density of questing nymphs (fitted values) depending on year, ground vegetation and season.

	Estimate (log)	Std Error	z-value	p-value
2018-2017	0.50	0.06	8.97	<0.001
2019-2017	0.63	0.09	6.72	<0.001
2019-2018	0.13	0.07	1.86	0.14
EV-BF	-0.08	0.11	-0.75	0.86
G-BF	-0.38	0.11	-3.49	0.00
M-BF	-0.68	0.16	-4.40	<0.001
G-EV	-0.30	0.06	-5.00	<0.001
M-EV	-0.60	0.12	-5.08	<0.001
M-G	-0.30	0.11	-2.78	0.02
May-July	0.22	0.06	3.82	<0.001
September-July	0.57	0.05	10.65	<0.001
September-May	0.35	0.05	6.52	<0.001

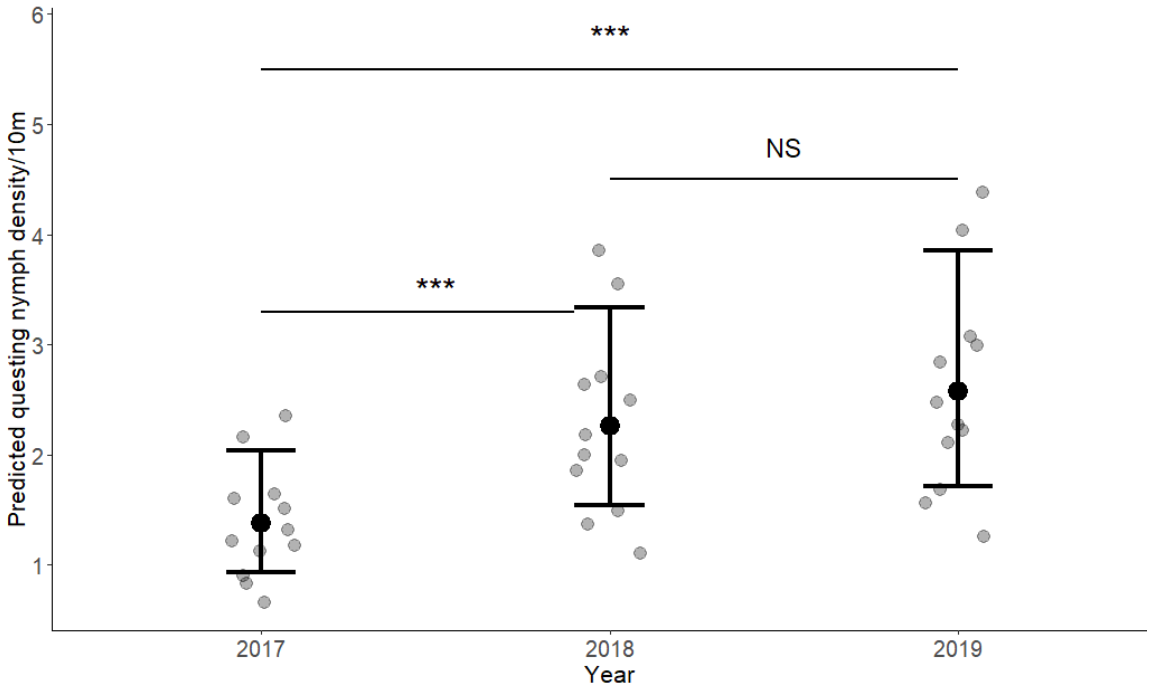


Figure A-S5.6: Predicted density of questing nymphs/10m in 2017, 2018 and 2019. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

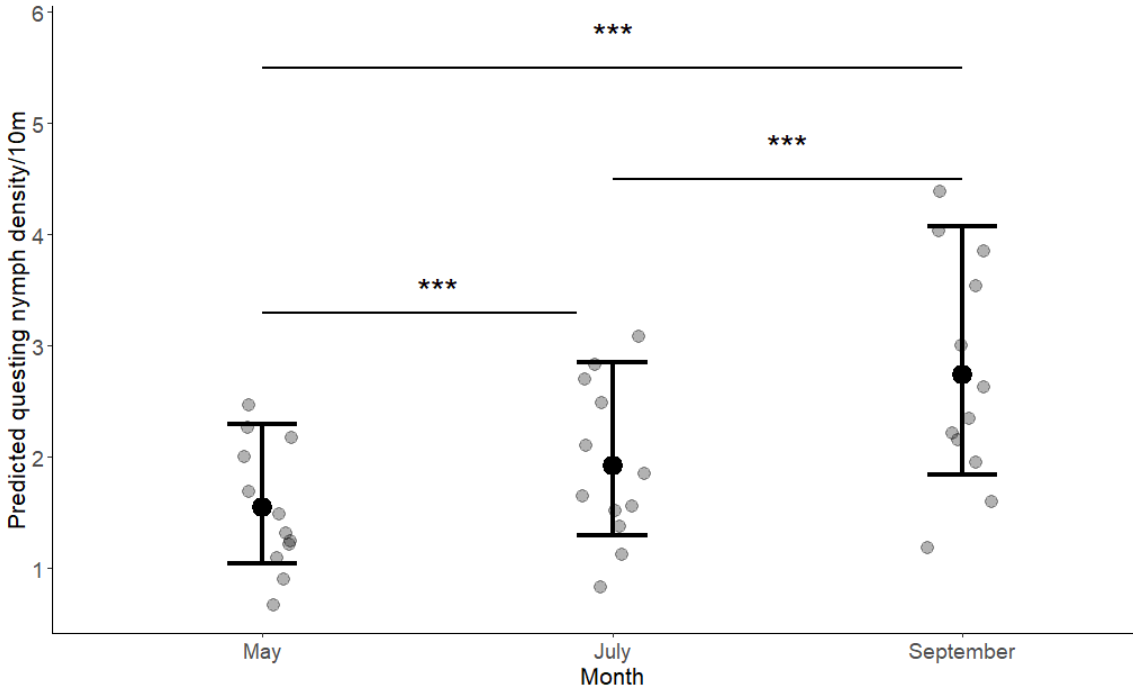


Figure A-S5.7: Predicted density of questing nymphs/10m in May, July and September. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

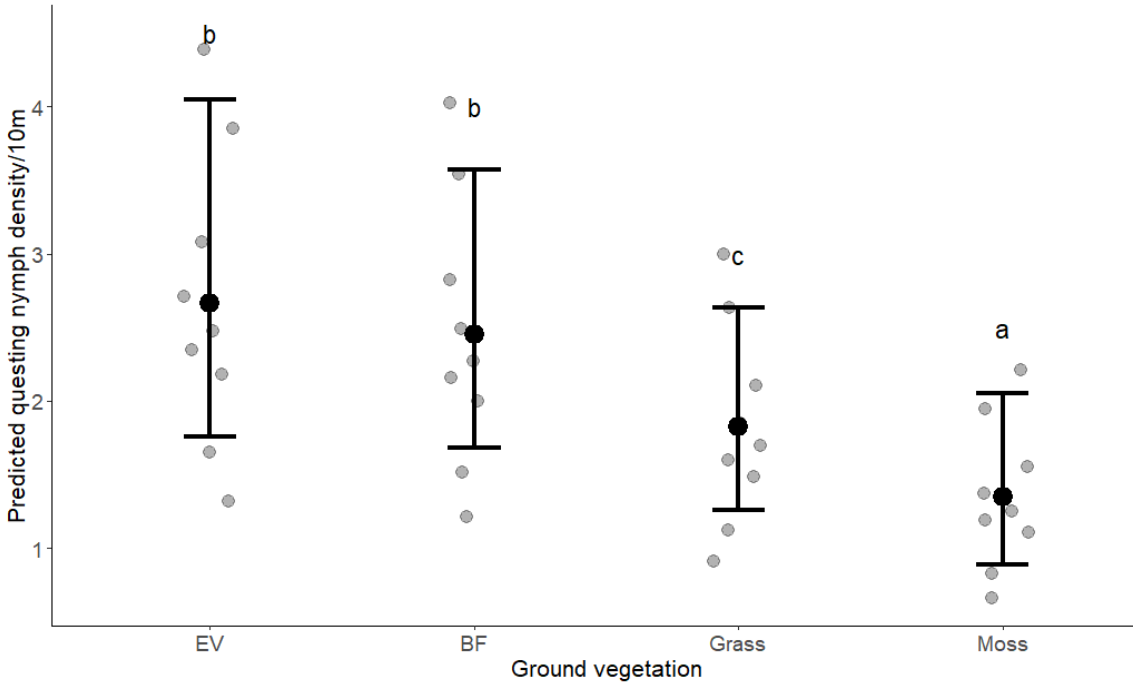


Figure A-S5.8: Predicted density of questing nymphs/10m depending on ground vegetation type. Different letters (a, b and c) show significant difference among groups based on Tukey HSD test.

S6: Deer as dilution hosts – NIP for *B. burgdorferi* s.l.

Table A-S6.1: Results from the selected models to explain what causes variations in the prevalence of *B. burgdorferi* s.l.

	Estimate (Log)	Std Error	z-value	p-value
Roe deer model				
Intercept	-2.77	0.28	-9.92	<0.001
Roe deer density	-0.12	0.03	-4.44	<0.001
Habitat: Deciduous	-1.62	0.58	-2.80	0.005
Habitat: Mixed	-0.71	0.38	-1.84	0.07
Red deer model				
Intercept	-4.51	0.27	-16.5	<0.001
Year: 2019	0.69	0.40	1.71	0.09

Table A-S6.2: Results from the Tukey HSD test representing the difference the average NIP (fitted values) depending on habitat type for roe deer.

	Estimate (log)	Std Error	z-value	p-value
Roe deer				
Deciduous-Coniferous	-1.62	0.58	-3.2	0.01
Mixed-Coniferous	-0.71	0.39	-1.9	0.15
Mixed-Deciduous	0.90	0.67	1.4	0.35

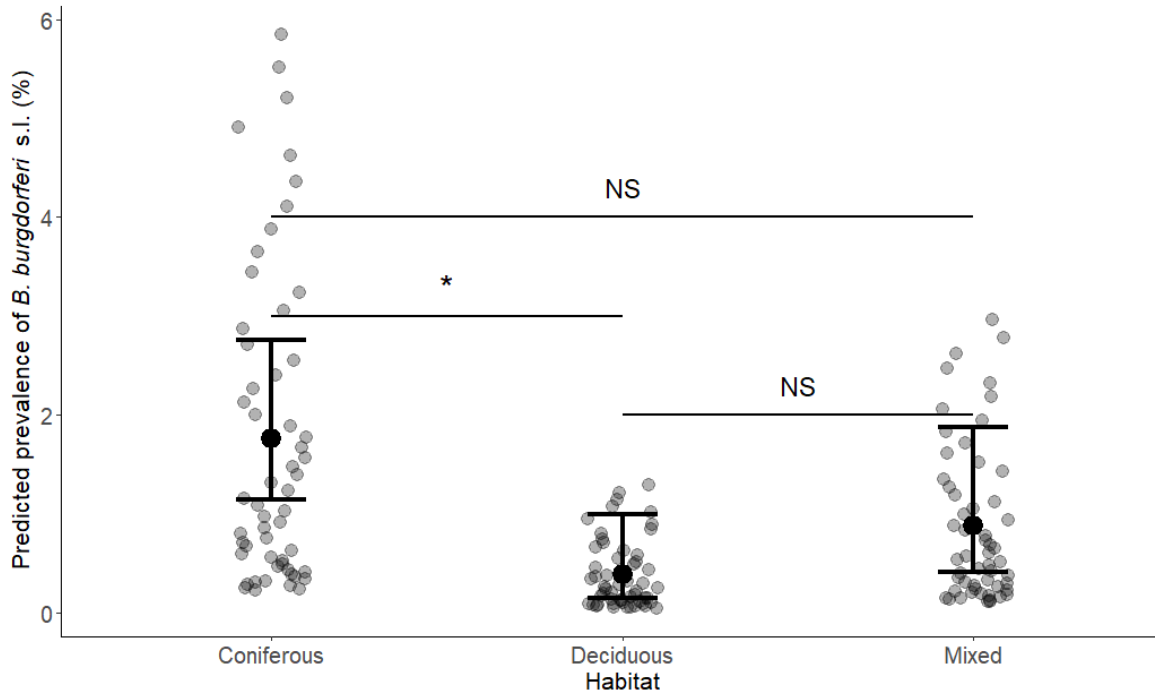


Figure A-S6.1: Predicted nymphal infection prevalence with *B. burgdorferi* s.l. (%) in Coniferous, deciduous and mixed woodlands. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

S7: Role of deer and small mammals – NIP for *B. afzelii*

Table A-S7.1: Results from the selected models to explain what causes variations in the prevalence of *B. afzelii* for models focussing on red and roe deer density.

Variable	Estimate (Log)	Std Error	z-value	p-value
Roe deer model				
Intercept	-4.05	0.63	-6.40	<0.001
Roe deer density year$_{t-1}$	-0.13	0.06	-2.12	0.03
Season: May	0.18	0.40	0.45	0.65
Season: September	0.86	0.37	2.33	0.02
Habitat: Deciduous	-1.86	0.79	-2.35	0.02
Habitat: Mixed	-0.88	0.54	-1.65	0.10
Small mammal index: Low	-0.13	0.74	-0.18	0.86
Roe deer * small mammal: Low	0.06	0.07	0.79	0.43
Red deer model				
Intercept	-5.89	0.50	-11.83	<0.001
Red deer density year$_{t-1}$	0.09	0.04	2.23	0.03
Season: May	0.17	0.41	0.42	0.67
Season: September	0.88	0.37	2.35	0.02
Small mammal index: Low	0.69	0.53	1.29	0.20
Red deer * small mammal: Low	-0.09	0.06	-1.34	0.18

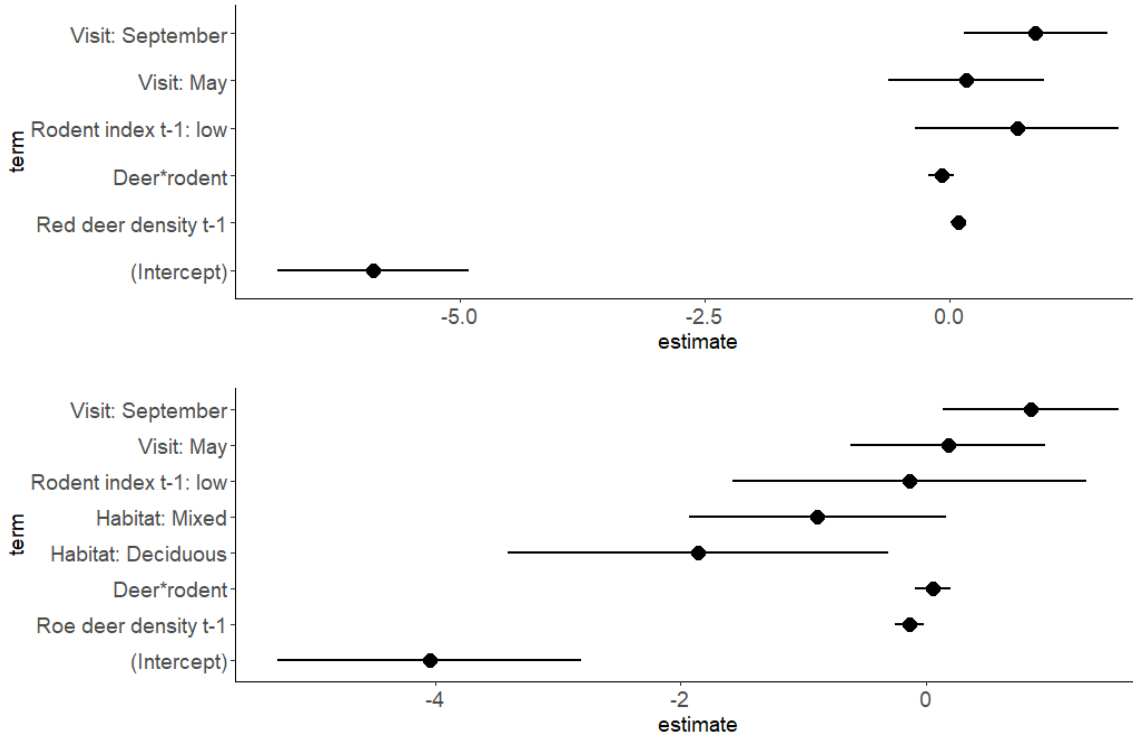


Figure A-S7.1: Effects of fixed variables on questing nymph abundance for the model focussing on roe deer (top) and red deer (bottom).

Table A-S7.2: Results from the Tukey HSD test representing the difference the average NIP (fitted values) depending on habitat type and season for roe deer and season for red deer.

	Estimate (log)	Std Error	z-value	p-value
Roe deer				
Deciduous-Coniferous	-1.86	0.79	0.5	0.05
Mixed-Coniferous	-0.88	0.54	2.3	0.21
Mixed-Deciduous	0.98	0.94	1.9	0.54
May-July	0.18	0.40	-2.3	0.89
September-July	0.86	0.37	-1.6	0.05
September-May	0.67	0.35	1.0	0.14
Red deer				
May-July	0.17	0.40	0.4	0.91
September-July	0.87	0.37	2.4	0.05
September-May	0.70	0.36	2.0	0.12

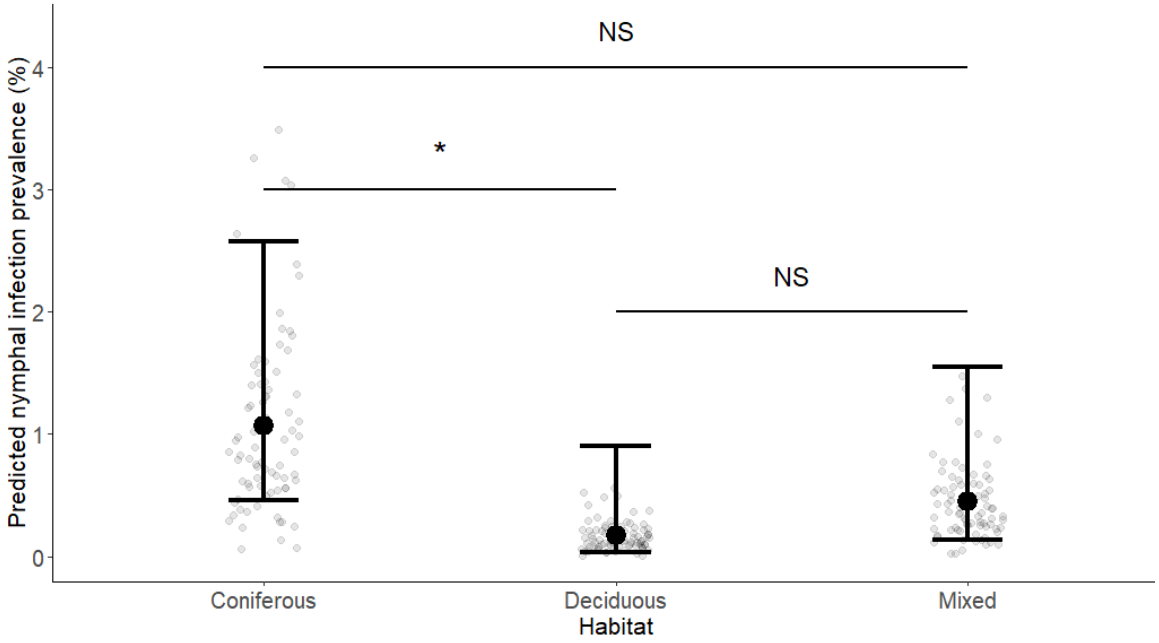


Figure A-S7.2: Predicted nymphal infection prevalence with *B. afzelii* (%) in Coniferous, deciduous and mixed woodlands. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

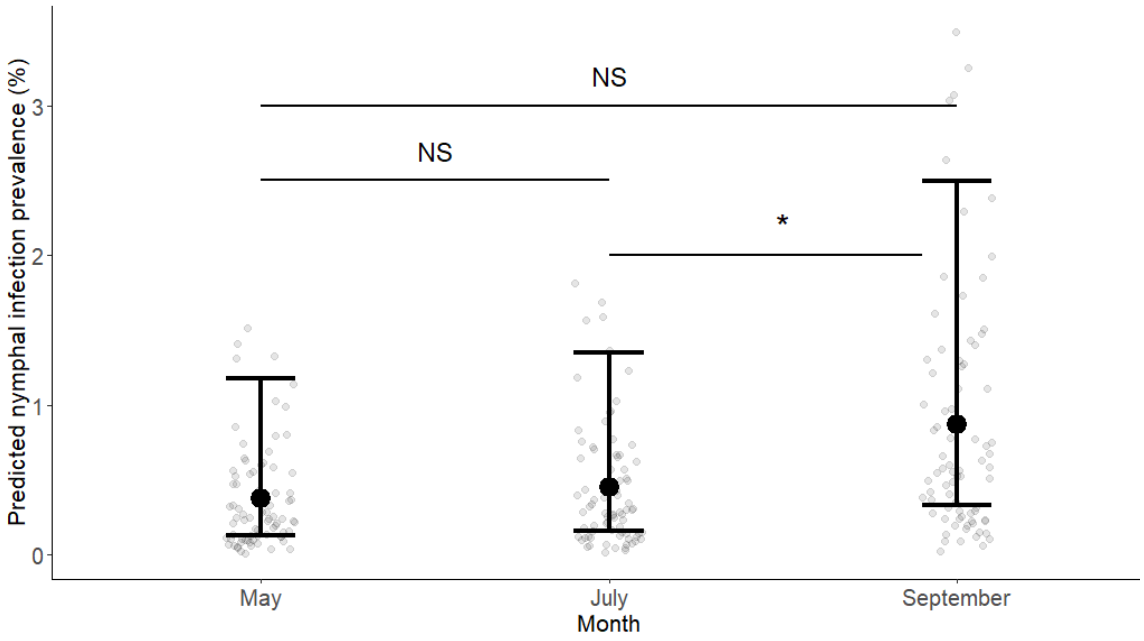


Figure A-S7.3: Predicted nymphal infection prevalence with *B. afzelii* (%) in May, July and September. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

S8: The effects of deer on DIN (*B. burgdorferi* s.l.)

Table A-S8.1: Results from the selected model to explain what causes variations in the density of nymphs infected with *B. burgdorferi* s.l.

Variable	Estimate (Log)	Std Error	z-value	p-value
Conditional model				
Intercept	-2.74	0.55	-5.01	<0.001
Habitat: Deciduous	-1.40	0.43	-3.26	0.001
Habitat: Mixed	-0.81	0.34	-2.37	0.02
Temperature	-0.12	0.03	-3.21	<0.001
Zero-inflated model				
Intercept	-0.47	0.27	-1.76	0.08

Table A-S8.2: Results from the Tukey HSD test representing the difference the average DIN (fitted values) depending on habitat type for red deer.

	Estimate (log)	Std Error	z-value	p-value
Deciduous-Coniferous	-1.40	0.43	-3.25	0.003
Mixed-Coniferous	-0.81	0.34	-2.37	0.04
Mixed-Deciduous	0.59	0.51	1.15	0.47

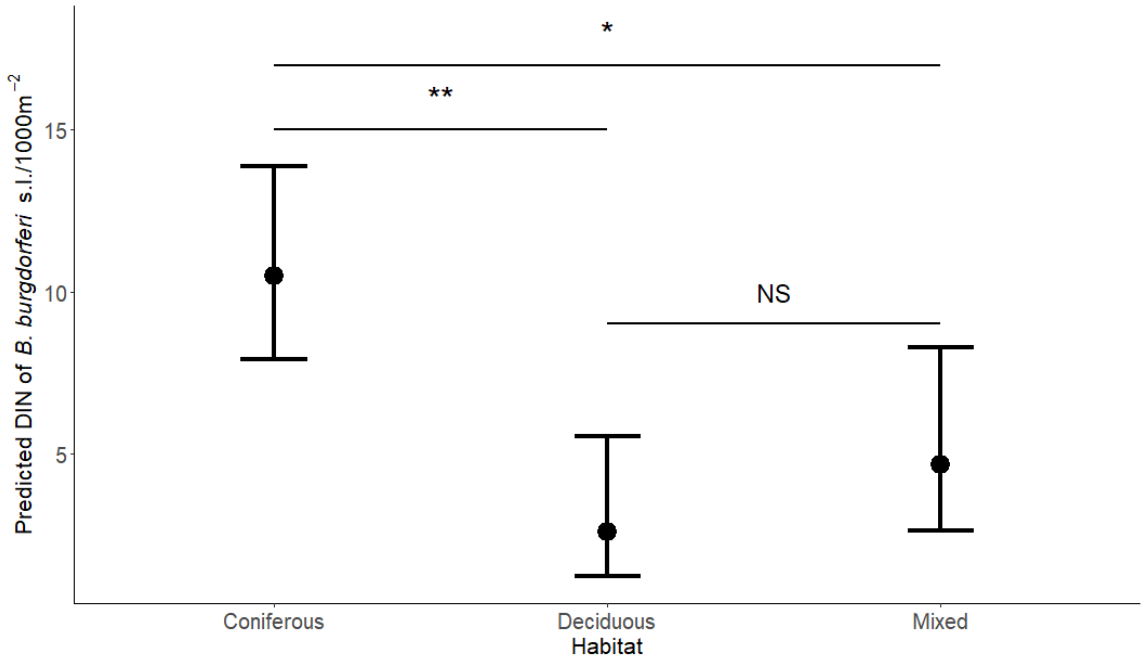


Figure A-S8.1: Predicted density of questing nymphs infected with *B. burgdorferi* s.l./1000m^{-2} in Coniferous, deciduous and mixed forests. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

S9: Effects of deer and small mammals on DIN (*B. afzelii*)

Table A-S9.1: Summary table from the conditional section of selected model showing which variables influence the density of nymphs infected with *B. afzelii* for the models focussing on roe and red deer.

Variable	Estimate (Log)	Std Error	z-value	p-value
Roe deer				
Conditional				
Intercept	-7.81	0.62	-12.52	<0.001
Roe deer density $year_{t-1}$	0.04	0.05	0.77	0.44
Season: May	1.02	0.45	2.25	0.02
Season: September	2.41	0.58	4.13	<0.001
Year: 2019	1.85	0.49	3.79	<0.001
Small mammal: low	-0.04	0.72	-0.05	0.96
Roe deer density * small mammal: low	0.06	0.08	0.78	0.44
Zero inflated				
Intercept	-0.35	0.32	-1.09	0.28
Red deer				
Conditional				
Intercept	-7.46	0.52	-14.37	<0.001
Red deer density $year_{t-1}$	0.07	0.03	2.36	0.02
Season: May	0.74	0.43	1.73	0.08
Season: September	2.31	0.55	4.24	<0.001
Year: 2019	1.13	0.48	2.34	0.02
Small mammal: low	-0.20	0.48	-0.42	0.67
Red deer density * small mammal: low	0.16	0.07	2.32	0.02
Zero inflated				
Intercept	-0.39	0.34	-1.14	0.25

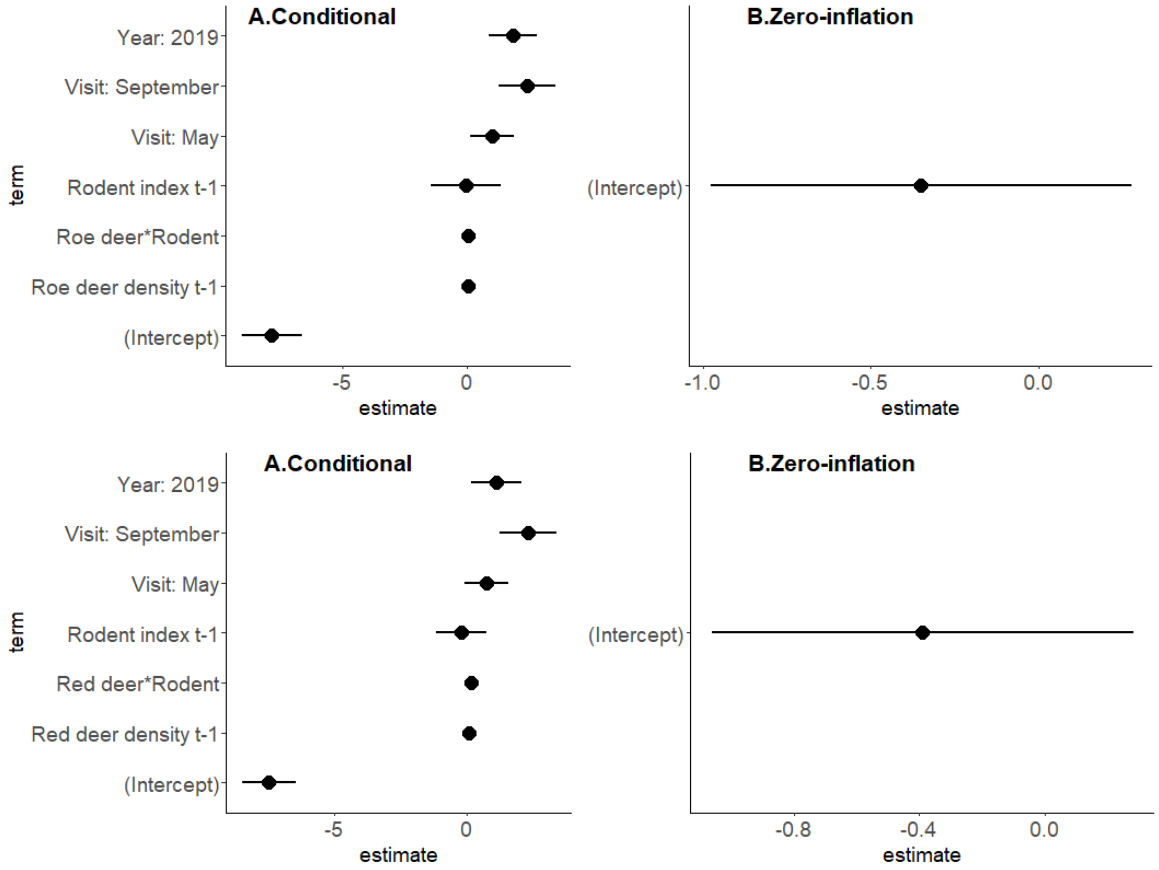


Figure A-S9.1: Effects of fixed variables on the density of questing nymphs infected with *B. afzelii* for the model focussing on roe deer (top) and red deer (bottom).

Table A-S9.2: Results from the Tukey HSD test representing the difference the average NIP (fitted values) depending on season for both roe and red deer.

	Estimate (log)	Std Error	z-value	p-value
Roe deer				
May-July	1.02	0.45	2.5	0.06
September-July	2.41	0.58	5.1	<0.001
September-May	1.39	0.53	2.6	0.02
Red deer				
May-July	0.75	0.43	1.7	0.19
September-July	2.28	0.54	4.2	<0.001
September-May	1.53	0.46	3.6	0.002

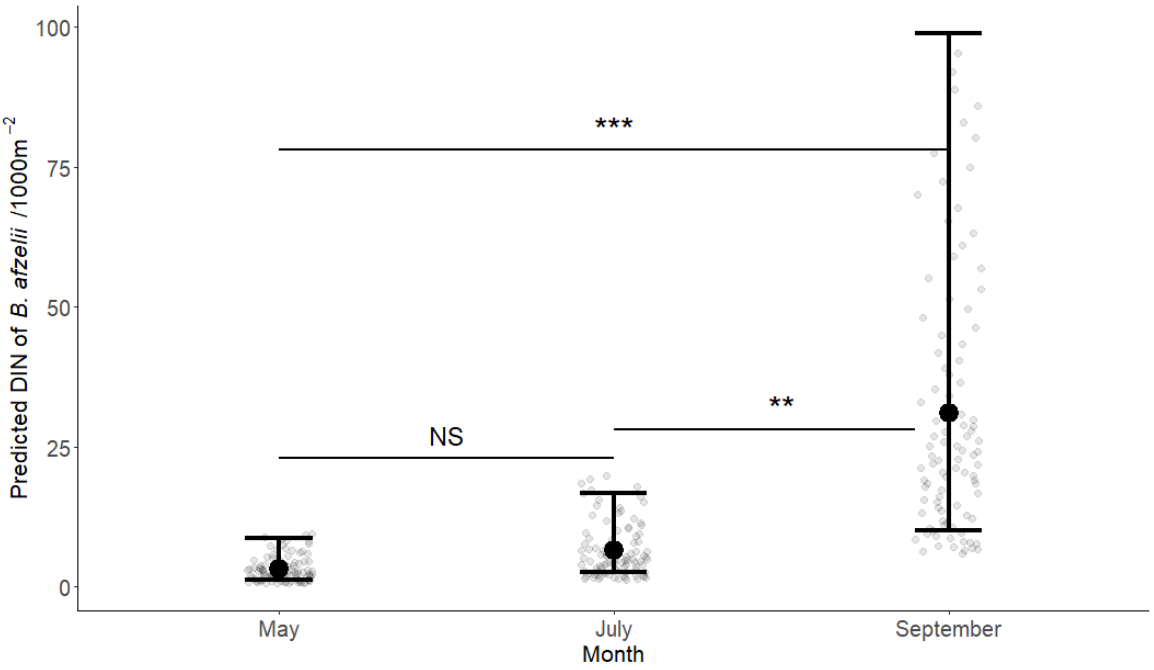


Figure A-S9.20.1: Predicted density of questing nymphs infected with *B.* afzelii/1000m^{-2} in May, July and September. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

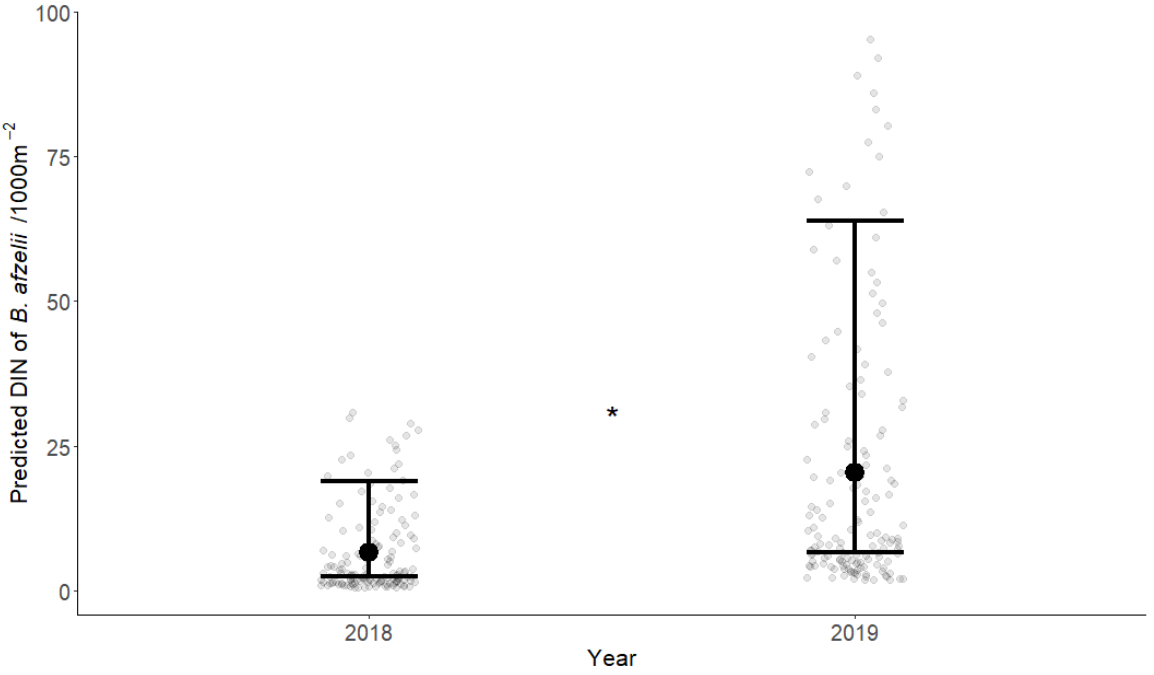


Figure A-S9.3: Plot representing the predicted density of questing nymphs infected with *B. afzelii* in 2018 and 2019. Statistical significance: *** p<0.001, ** p<0.01, * p<0.05, NS p>0.05 from Tukey HSD tests.

S10: Factors influencing Small mammal abundance

Table A-S10.1: Results from the selected model to explain what causes variations in small mammal abundance for models focussing on red and roe deer density.

	Estimate (Log)	Std Error	z-value	p-value
Roe deer model				
Intercept	-3.96	0.54	-7.31	<0.001
Vegetation: EV	0.08	0.65	0.12	0.90
Vegetation: Grass	-2.11	0.71	-2.97	0.003
Vegetation: Mixed	-0.60	0.76	-0.78	0.44
Vegetation: Moss	-0.16	0.87	-0.19	0.85
Red deer model				
Intercept	-3.87	0.38	-10.14	<0.001
Red deer density year$_t$	-0.15	0.06	-2.77	0.01
Vegetation: EV	0.84	0.49	1.69	0.09
Vegetation: Grass	-1.60	0.55	-2.90	0.004
Vegetation: Mixed	0.31	0.62	0.50	0.62
Vegetation: Moss	-0.17	0.62	-0.27	0.79

Table A-10.2: Results from the Tukey HSD test representing the difference the average number of individuals captured per 100 trap nights (fitted values) depending ground vegetation for both roe and red deer.

	Estimate (log)	Std Error	p-value
Roe deer			
EV-BFG	0.08	0.65	1.00
Grass-BFG	-2.11	0.71	0.02
Mixed-BFG	-0.60	0.76	0.93
Moss-BFG	-0.16	0.87	1.00
Grass-EV	-2.19	0.57	<0.001
Mixed-EV	-0.68	0.64	0.82
Moss-EV	-0.24	0.77	1.00
Mixed-Grass	1.51	0.69	0.18
Moss-Grass	1.94	0.82	0.12
Moss-Mixed	0.43	0.87	0.99
Red deer			
EV-BFG	0.84	0.49	0.43
Grass-BFG	-1.60	0.55	0.03
Mixed-BFG	0.31	0.62	0.99
Moss-BFG	-0.17	0.62	1.00
Grass-EV	-2.44	0.47	<0.001
Mixed-EV	-0.53	0.48	0.80
Moss-EV	-1.00	0.58	0.41
Mixed-Grass	1.91	0.58	0.01
Moss-Grass	1.43	0.63	0.15
Moss-Mixed	-0.48	0.69	0.96

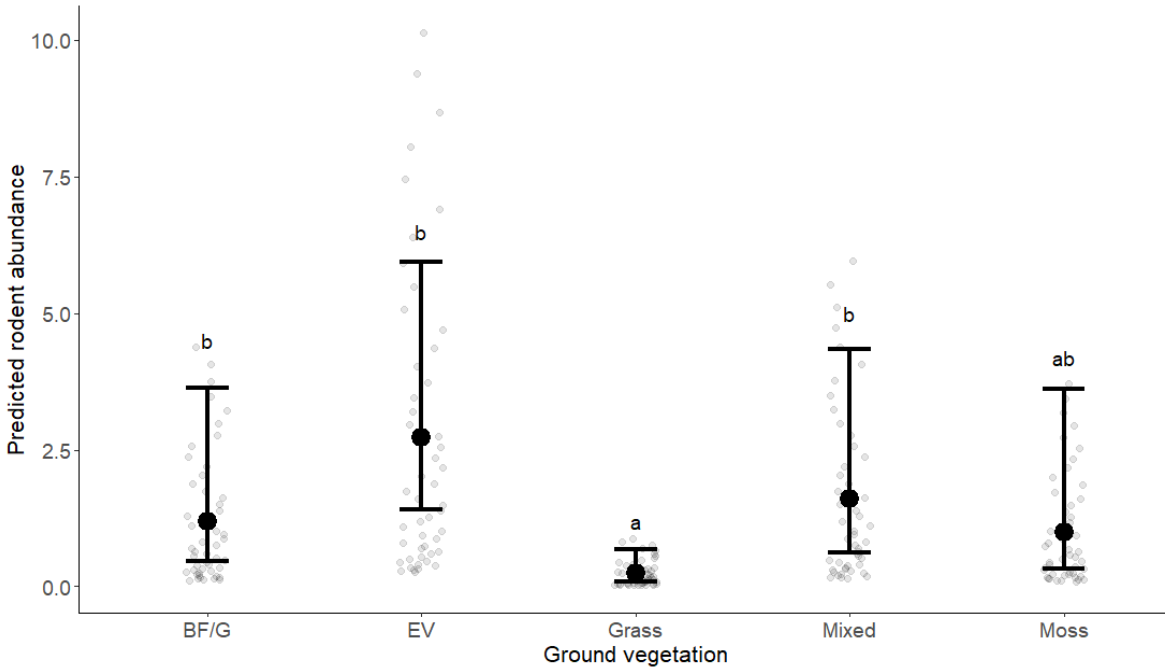


Figure A-10.1: Predicted small mammal abundance/100TN depending on ground vegetation type. Different letters (a, b and c) show significant difference among groups based on Tukey HSD test.

Appendix B: Supplementary materials for Chapter 3

S1: Number of trees measured

Table B-S1.1: Number of birch and pine trees measured in deer exclusion and high deer density plots between 2013 and 2014.

Plots	Tree	May 2013	July 2013	September 2013	May 2014	July 2014	September 2014
High deer density	Birch	5	30	0	40	0	40
	Pine	0	0	0	38	0	40
Deer exclusion	Birch	0	10	0	40	0	40
	Pine	0	10	0	32	0	40

S2: Questing nymph abundance model summary

Table B-S2.1: Results from the selected hurdle model to explain what causes variations in questing nymph abundance.

	Estimate (Log)	Std Error	z-value	p-value
Conditional model				
Intercept	2.44	0.10	25.07	<0.001
Plot treatment: deer exclusion	-2.32	0.10	-23.04	<0.001
Month: May	-0.25	0.08	-2.97	0.003
Month: September	-0.30	0.07	-4.44	<0.001
Year: 2014	0.05	0.06	0.80	0.43
Year: 2018	0.89	0.08	11.12	<0.001
Year: 2019	-0.24	0.10	-2.50	0.01
Habitat: heather	-0.78	0.11	-7.26	<0.001
Habitat: pine	-0.15	0.10	-1.45	0.15
Relative humidity	-0.18	0.03	-5.46	<0.001
Ground wet: yes	-0.48	0.18	-2.72	0.01
Temperature	-0.11	0.04	-2.60	0.01
Zero-inflation model				
Intercept	-2.81	0.20	-13.81	<0.001
Plot treatment: deer exclusion	3.55	0.15	24.07	<0.001
Month: May	0.57	0.19	3.03	0.002
Month: September	-0.23	0.18	-1.32	0.19
Habitat: heather	0.65	0.15	4.45	<0.001
Habitat: pine	-0.01	0.14	-0.06	0.95
Year: 2014	-0.28	0.15	-1.91	0.06
Year: 2018	-0.71	0.20	-3.61	<0.001
Year: 2019	-0.75	0.21	-3.50	<0.001
Temperature	-0.21	0.08	-2.59	0.01
Ground wet: yes	2.13	0.34	6.19	<0.001

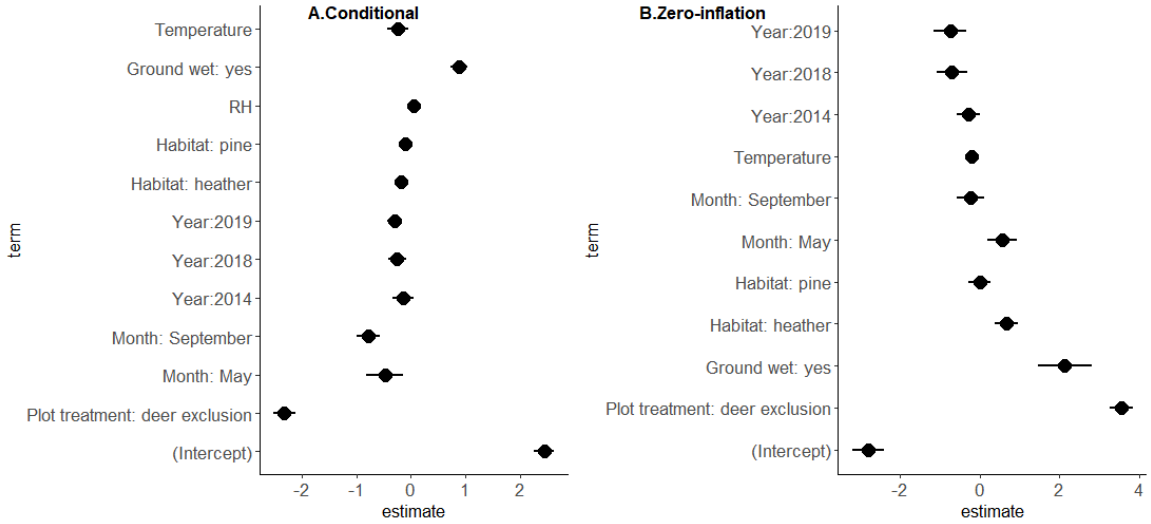


Figure B-S2.1: Graph showing the effects of the parameters on the response variable.

S3: Questing nymph abundance - post hoc Tukey tests

Table BS3.1: Results from the Tukey HSD test representing the difference in the average density of questing nymphs (fitted values) depending on year, habitat type and month.

	Estimate (log)	Std Error	z-value	p-value
2014-2013	0.05	0.06	0.80	0.85
2018-2013	0.89	0.08	11.12	<0.001
2019-2013	-0.24	0.10	-2.50	0.06
2018-2014	0.84	0.08	10.89	<0.001
2019-2014	-0.29	0.10	-2.94	0.02
2019-2018	-1.13	0.11	-10.26	<0.001
Heather – Birch	-0.78	0.11	-7.26	<0.001
Pine – Birch	-0.15	0.10	-1.45	0.32
Pine – Heather	0.64	0.11	5.95	<0.001
May-July	-0.25	0.08	-2.97	0.01
September-July	-0.30	0.07	-4.44	<0.001
September-May	-0.04	0.07	-0.60	0.82

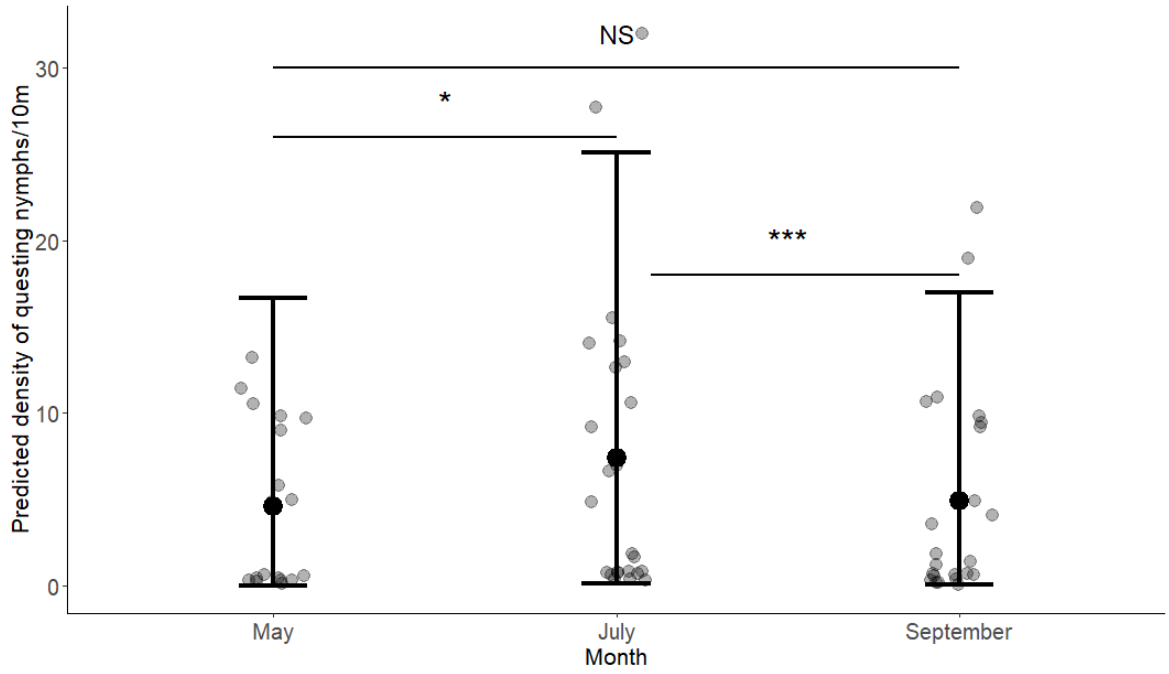


Figure B-S3.1: Predicted density of nymphs/10m depending on month. Error bars represent 95% CI. Statistical significance from Tukey tests: NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

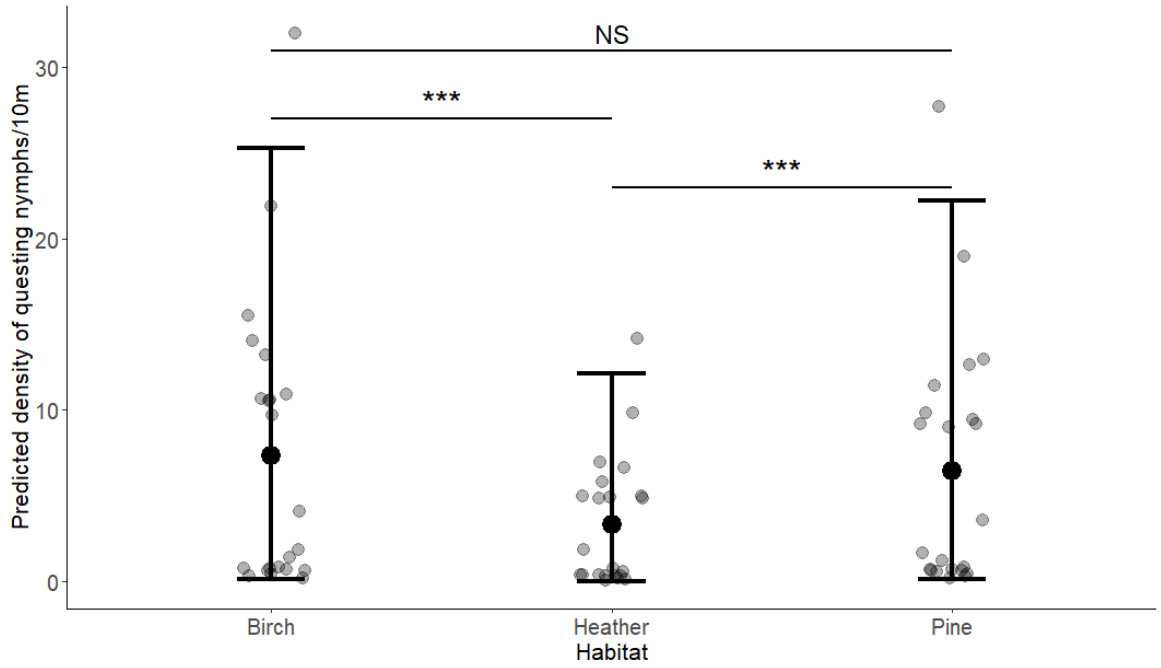


Figure B-S3.2: Predicted density of nymphs/10m depending on habitat. Error bars represent 95% CI. Statistical significance from Tukey tests: NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

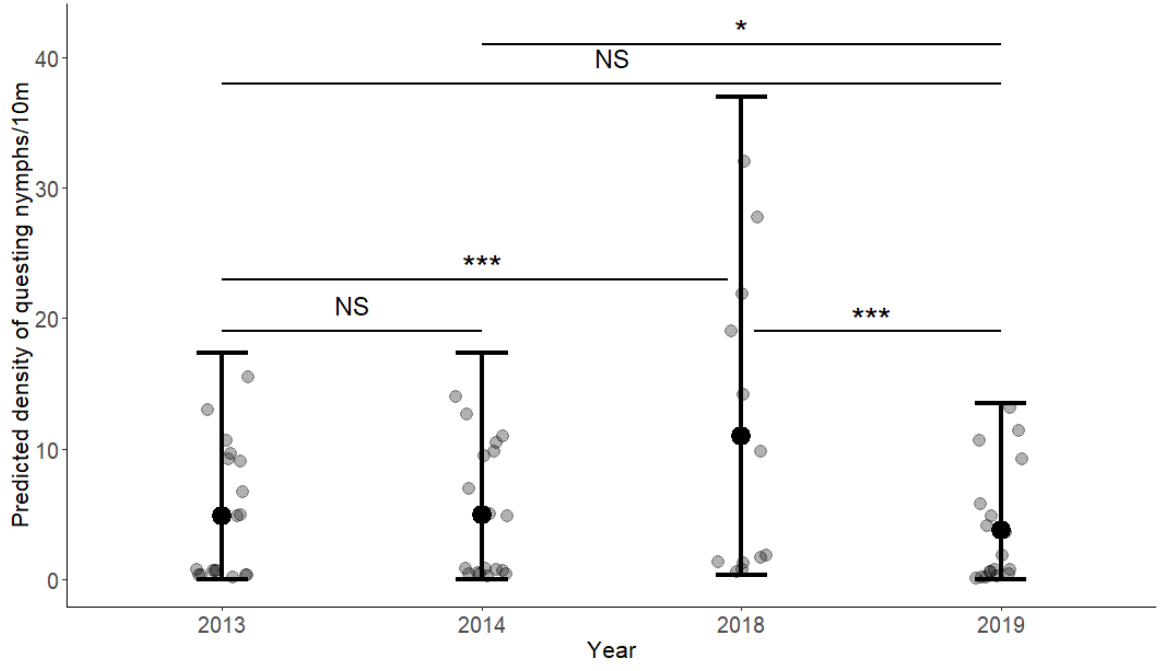


Figure B-S3.3: Predicted density of nymphs/10m depending on year. Error bars represent 95% CI. Statistical significance from Tukey tests: NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

S4: Summary of NIP and DIN data

Table B-S4.1: Detailed summary table of how many questing nymphs were tested in deer exclusion and high deer density plots in 2013, 2014, 2018 and 2019 and the proportion carrying *Borrelia burgdorferi* s.l. (NIP) as well as the 4 genospecies (*B. afz: B. afzelii*, *B. gar: B. garinii*, *B. val:B. valaisiana*, *B.b.* s.s: *B. burgdorferi* s.s.). DIN represents the density of infected nymph per $10m^{-2}$.

Year	Plot treatment	DIN	Total NIP (%)	*B. afz.* (%)	*B. gar.* (%)	*B. val.* (%)	*B. b.* s.s (%)
2013	Deer exclusion	0.01	1.92 (2/104)	1.92	0	0	0
	High deer density	0.56	0.56 (1/177)	0	0	0.56	0
2014	Deer exclusion	0.02	4.23 (9/213)	3.76	0.47	0	0
	High deer density	0	0 (0/120)	0	0	0	0
2018	Deer exclusion	0.01	0.72 (1/139)	0.72	0	0	0
	High deer density	0.06	0.24 (1/422)	0.24	0	0	0
2019	Deer exclusion	0.02	3.10 (4/129)	3.10	0	0	0
	High deer density	0.50	1.78 (7/323)	1.78	0	0	0
	Deer exclusion		**2.74 (16/585)**	**2.56 (15/585)**	**0.17 (1/585)**	**0**	**0**
	High deer density		**0.86 (9/1042)**	**0.77 (8/1042)**	**0.10 (1/1042)**	**0.10 (1/1042)**	**0**

S5: NIP model (*B. burgdorferi* s.l.)

Table B-S5.1: Summary table from the selected model showing which variables were associated with the prevalence of *B. burgdorferi* s.l. in questing nymphs.

	Estimate (log)	Std Error	z-value	p-value
Intercept	-6.20	0.96	-6.47	<0.001
Plot treatment: deer exclusion	0.77	0.52	1.48	0.14
Month: May	-1.07	0.83	-1.30	0.20
Month: September	0.90	0.50	1.79	0.07
Habitat: Heather	1.05	0.70	1.49	0.14
Habitat: Pine	1.45	0.62	2.34	0.02
Year: 2014	0.72	0.74	0.97	0.33
Year: 2018	-1.03	0.95	-1.09	0.28
Year: 2019	1.02	0.70	1.46	0.14

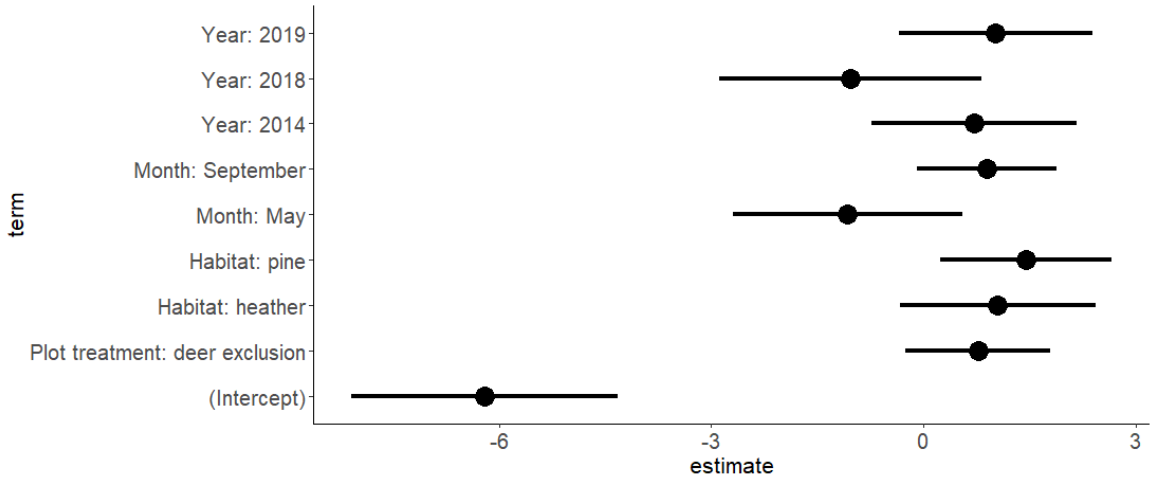


Figure B-S5.1: Effects of the parameters on the response variable.

Table B-S5.2: Results from the Tukey HSD test representing the difference in the average prevalence of *B. burgdorferi* s.l. (fitted values) depending on year, habitat and month.

	Estimate (log)	Std Error	z-value	p-value
2014-2013	0.72	0.74	0.97	0.83
2018-2013	-1.03	0.95	-1.09	0.83
2019-2013	1.02	0.70	1.46	0.58
2018-2014	-1.75	0.86	-2.03	0.21
2019-2014	0.30	0.57	0.53	0.83
2019-2018	2.05	0.81	2.54	0.07
Heather – Birch	1.05	0.70	1.50	0.27
Pine – Birch	1.45	0.62	2.34	0.06
Pine – Heather	0.40	0.54	0.74	0.46
May-July	-1.07	0.83	-1.30	0.20
September-July	0.90	0.50	1.79	0.15
September-May	1.97	0.80	2.47	0.04

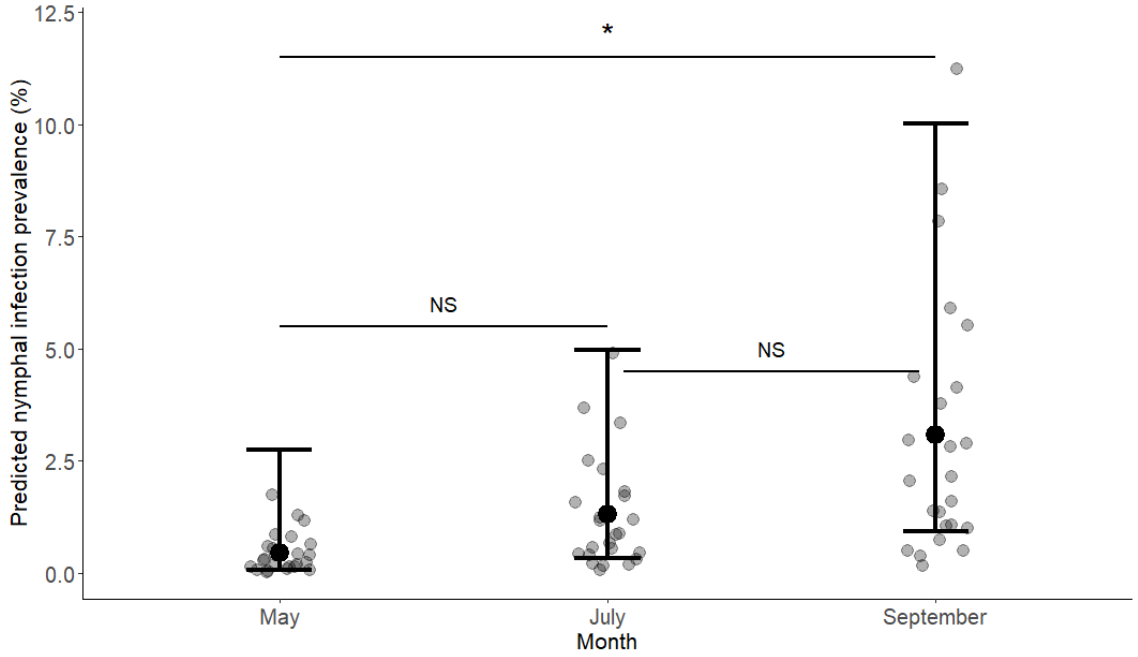


Figure B-S5.2: Predicted nymphal infection prevalence with *B. burgdorferi* s.l. (%) depending on month. Error bars represent 95% CI. Statistical significance from Tukey tests: NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

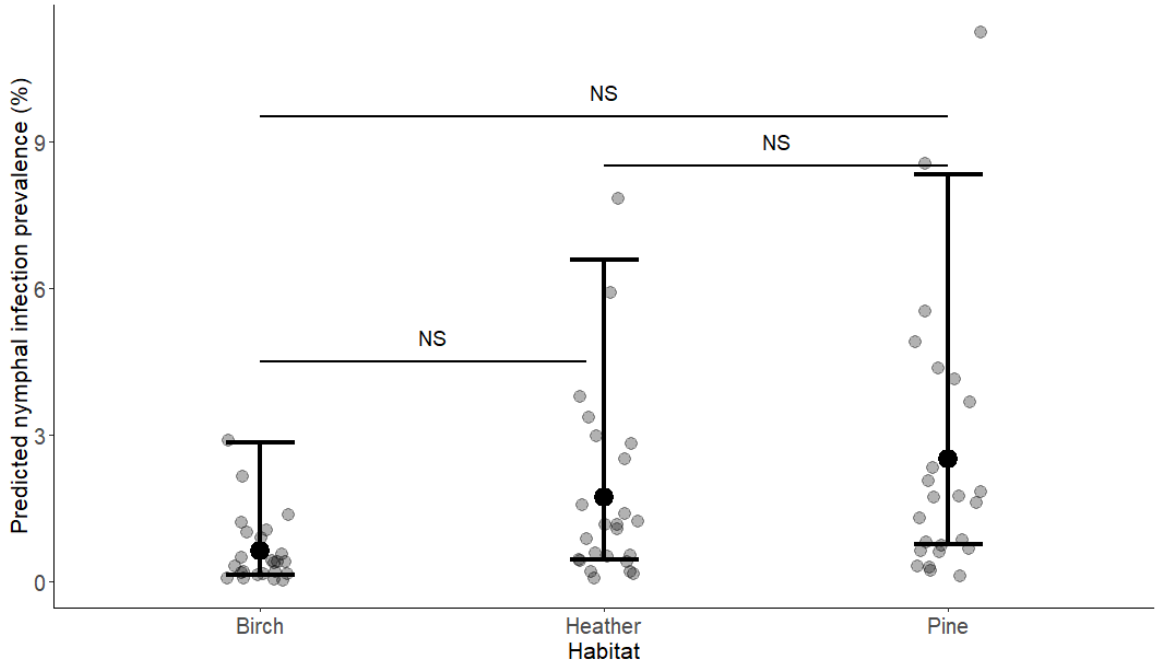


Figure B-S5.3: Predicted nymphal infection prevalence with *B. burgdorferi* s.l. (%) depending on habitat. Error bars represent 95% CI. Statistical significance from Tukey tests: NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

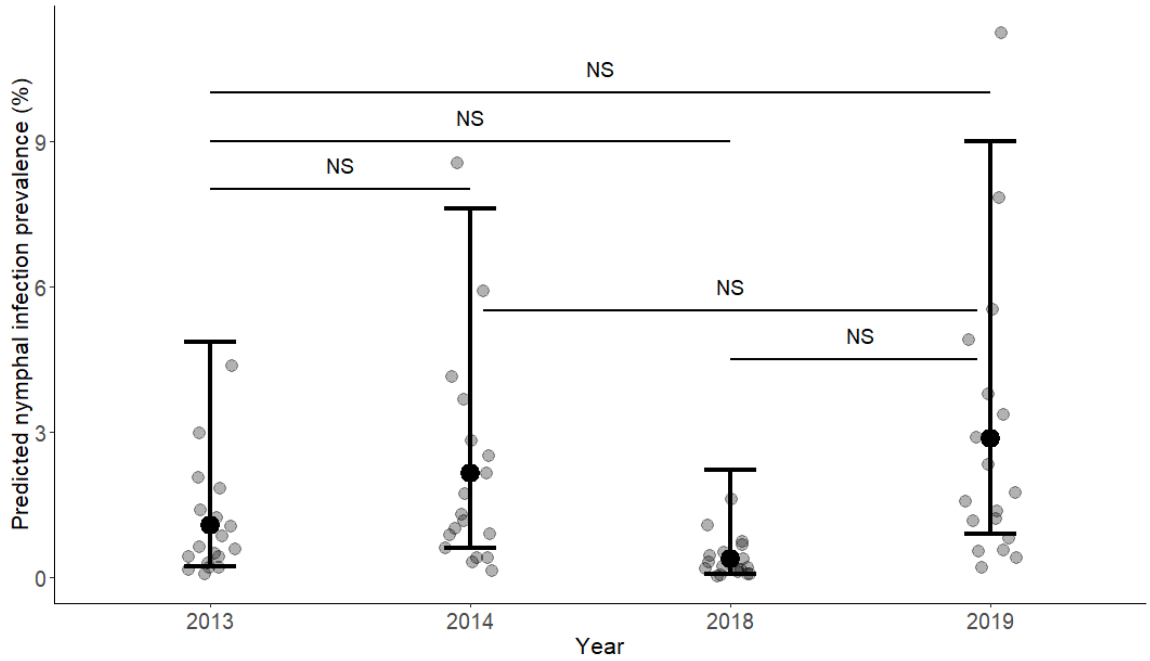


Figure B-S5.4: Predicted nymphal infection prevalence with *B. burgdorferi* s.l. (%) depending on year. Error bars represent 95% CI. Statistical significance from Tukey tests: NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

S6: GLMM for *B. afzelii* NIP

The mixed effect model used to investigate the effect of fencing, habitat, month and year on *B. burgdorferi* s.l. infection prevalence in questing nymphs was conducted for *B. afzelii* infections only. As seen from Table B-S6.1Table , results are similar to the other model.

Table B-S6.1: Results from the selected model to explain what causes variations in the prevalence of *B. afzelii*. ΔAICc indicates the increase in AICc after dropping each variable from the model.

	Estimate (Log)	Std Error	z-value	p-value	ΔAICc
Intercept	-6.58	1.12	-5.88	<0.001	
Plot treatment: deer exclusion	0.80	0.57	1.40	0.16	0.5
Month: May	-0.88	0.82	-1.07	0.28	5.8
Month: September	1.07	0.51	2.08	0.04	
Habitat: heather	0.90	0.70	1.28	0.20	1.9
Habitat: pine	1.38	0.61	2.27	0.02	
Year: 2014	1.00	0.82	1.21	0.22	3.6
Year: 2018	-0.63	1.01	-0.63	0.53	
Year: 2019	1.40	0.79	1.77	0.08	

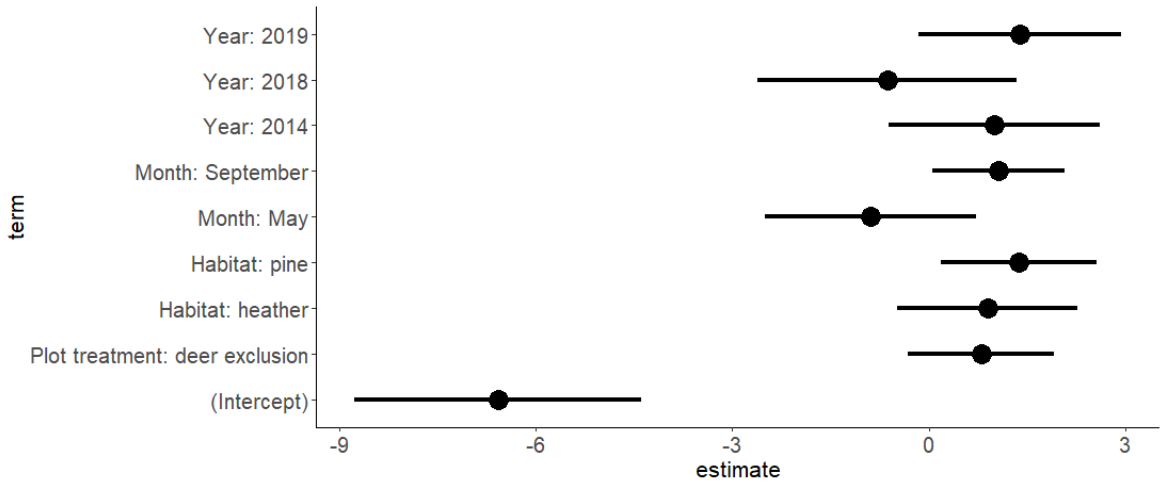


Figure B-S6.1: Graph showing the effects of the parameters on the response variable.

S7: DIN for *B. burgdorferi* s.l. model summary

Table B-S7.1: Results from the selected model to explain what causes variations the density of nymphs infected with *B. burgdorferi s.l.*

	Estimate (Log)	Std Error	z-value	p-value
Conditional model				
Intercept	-5.85	0.75	-7.84	<0.001
Plot treatment: deer exclusion	-1.76	0.28	-6.26	<0.001
Habitat: Heather	0.05	0.42	0.12	0.91
Habitat: Pine	0.83	0.39	2.12	0.03
Temperature	0.10	0.05	2.27	0.02
Zero-inflation model				
Intercept	-8.53	1.10	-7.79	<0.001
Ground wet: no	3.12	1.15	2.73	0.006

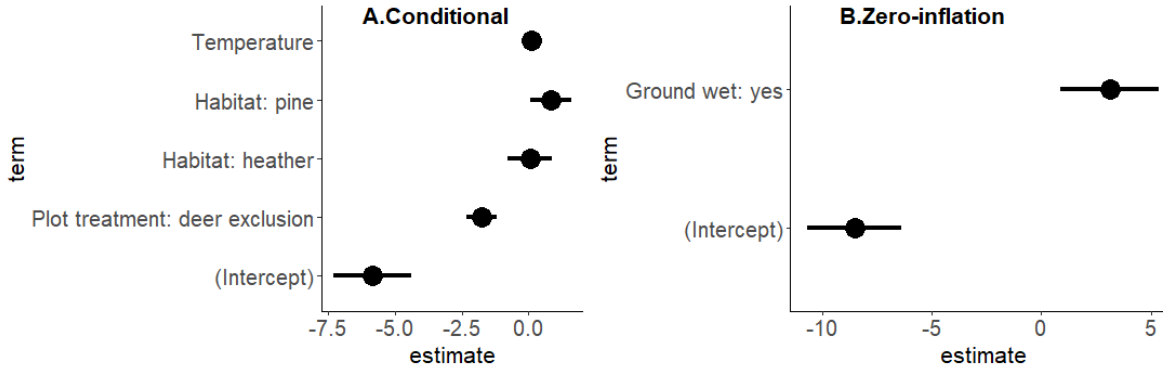


Figure B-S7.1: Graph showing the effects of the parameters on the response variable.

S8: DIN post hoc Tukey tests

Table B-S8.1: Results from the Tukey HSD test representing the difference in the average density of nymphs infected by B. burgdorferi s.l. per 1000m-2 (fitted values) depending on habitat type.

	Estimate (log)	Std Error	z-value	p-value
Heather - Birch	-0.16	0.40	-0.41	0.91
Pine - Birch	0.71	0.35	2.01	0.10
Pine - Heather	0.88	0.26	3.42	0.002

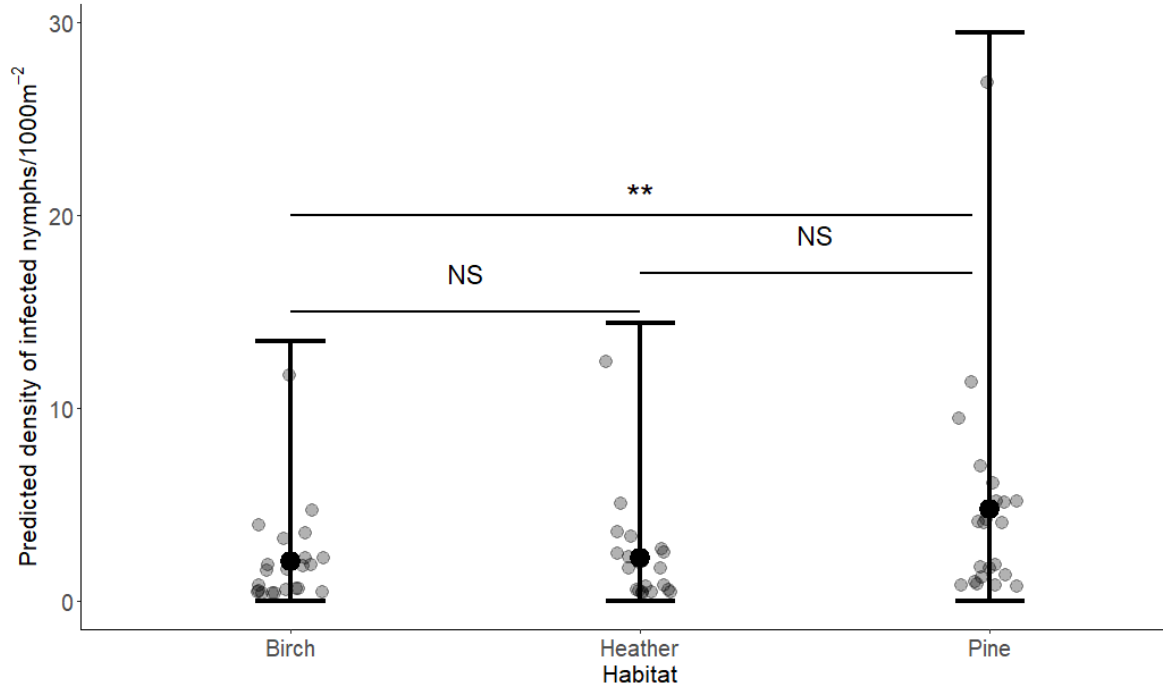


Figure B-S8.1: Plot showing the predicted density of nymphs infected with *B. burgdorferi* s.l./1000m^{-2} depending on habitat. Error bars represent 95% CI. Statistical significance from Tukey tests: NS: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

Appendix C: Supplementary materials for Chapter 4

S1: Individual tick burden on wood mice depending on time between two recaptures

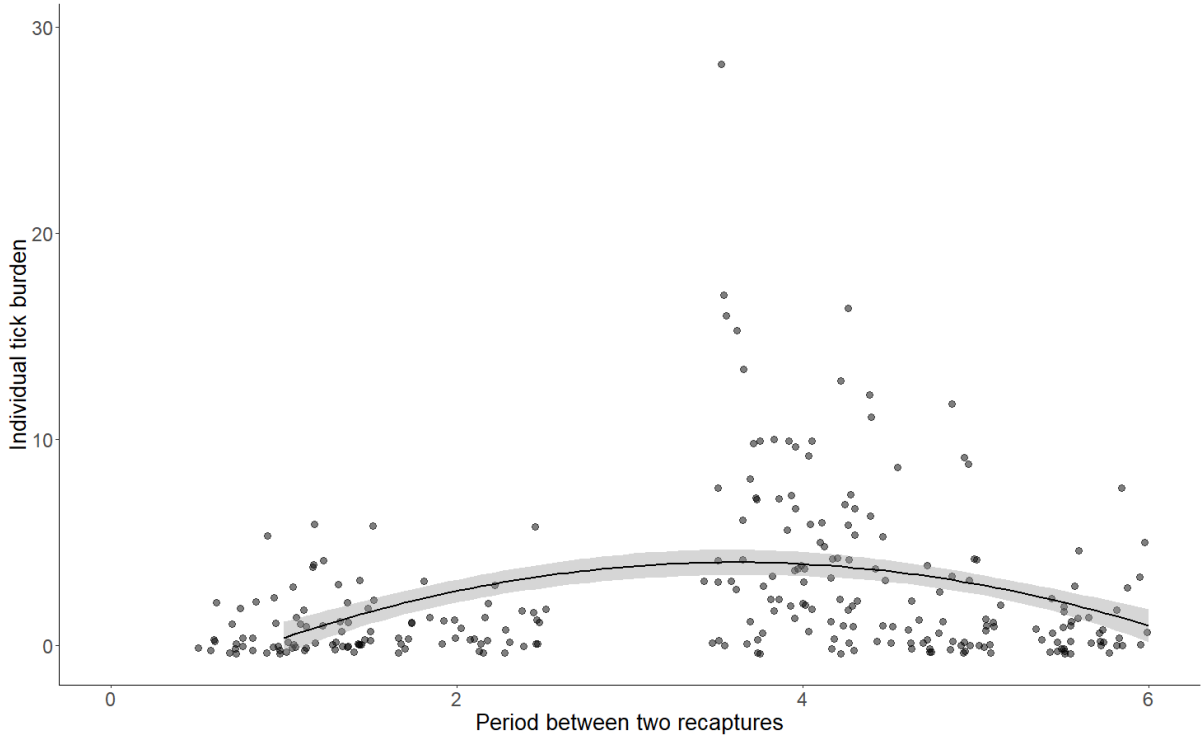


Figure C-S1.1: Scatter plot showing individual tick burden on wood mice depending on period between two recaptures of the same individual.

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