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Ticks and Tick-borne Diseases 16 (2025) 102541

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

The role of large ungulate grazers on *Ixodes ricinus* and tick-borne pathogens in the New Forest - a case study for future rewilded landscapes

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ARTICLE INFO

Keywords:
Conservation grazing
Tick-borne fever
Ticks
Borrelia
Anaplasma
Rewilding

ABSTRACT

Large ungulate grazers can manage habitats via conservation grazing, a practice using livestock to control vegetation growth, which has many ecological benefits but has the potential to provide additional hosts for ticks and consequently have an impact on tick-borne disease risk. Cattle and sheep are suspected to be transmission hosts for several tick-transmitted pathogens, so the presence of livestock could increase disease hazard. However, some ungulate species do not transmit other pathogens such as *Borrelia burgdorferi* sensu lato (s.l.), so conservation grazing could reduce prevalence of these pathogens, and thus environmental disease hazard, by diverting ticks from feeding on transmission hosts. To better understand these dynamics, we used a paired experiment in the New Forest in southern England. Questing ticks were collected at 20 sites between 2021 and 2023. Ten sites were inside "inclosures" (New Forest term for fenced woodlands to exclude livestock) and the remaining ten were not fenced, which permitted livestock grazing. Grazing led to significantly shorter ground vegetation and fewer questing *Ixodes ricinus* nymphs. We tested 2974 nymphs for multiple pathogens and determined there were no significant differences in nymphal infection prevalence or density of infected nymphs for *B. burgdorferi* s.l. and *Anaplasma phagocytophilum* between sites. However, we found that the density of infected nymphs for *Borrelia garinii* and *Borrelia valaisiana* was lower where there was grazing. In this study, we show that conservation grazing by ponies and cattle could lower tick density, probably by affecting the vegetation understory, and could potentially lower disease hazard for some genospecies of *B. burgdorferi* s.l. but not *A. phagocytophilum*.

1. Introduction

The concept of rewilding, which emerged in the 1990s, promotes increasing biodiversity and restoring natural processes in ecosystems by reducing human influence (Soulé and Noss, 1998). This approach to land management and conservation can involve actions such as the increase of habitat connectivity, wetland restoration, and species reintroduction. Some of these concepts and their impact on pathogen vectors have been investigated in relation to ticks and agri-environment schemes (Medlock et al., 2020) and mosquito abundance in response to wetland creation and management (Medlock and Vaux, 2015a; 2015b). Southern England now has a number of rewilding projects, some of which have been ongoing for 20 years. Actions have included improvement of natural processes by restoring river systems, improving woodland connectivity, allowing hedgerows and pastures to develop according to natural succession processes, and using native livestock breeds to manage pastures. These changes in land use have raised the question of whether rewilded countryside areas involving the introduction of large ungulate grazers will increase tick abundance and increase the risk of tick-borne diseases.

The United Kingdom (UK) government recently introduced a financial incentive to encourage farmers and landowners to undertake rewilding projects (DEFRA, 2023; Harvey, 2022). One of the actions that can be undertaken is conservation grazing through the use of livestock to control vegetation growth in fields and woodlands. Conservation grazing has many benefits, including vegetation growth control, development of structural heterogeneity (Mitchell and Kirby, 1990; Rook and Tallowin, 2003), and increased plant species richness (Lilleeng et al.,

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https://doi.org/10.1016/j.ttbdis.2025.102541
Received 1 April 2025; Received in revised form 4 August 2025; Accepted 19 August 2025
Available online 29 August 2025

2016; Lyons et al., 2017). Grazing can also help prevent the regeneration and spread of non-native invasive species (Dornbusch et al., 2020; Öllerer et al., 2019), provided the species is susceptible to grazing (Kimball and Schiffman, 2003). Grazing by cattle has been shown to have positive effects on a range of invertebrates including butterfly communities in calcareous pastures (Bussan, 2022; Joubert et al., 2016).

Conversely, conservation grazing can reduce species richness in small mammal communities, leaving a few, more tolerant species to thrive (Evans et al., 2006; Putman, 1986; Putman et al., 1989; Schieltz and Rubenstein, 2016; van Wieren and Bakker, 2016). Grazing pressure can also impact tree regeneration by up to 40 % (Armstrong et al., 2003; Putman et al., 1989) while trampling and grazing can also lead to an increase in bare soil cover affecting invertebrate and fungi communities (Alexander et al., 2023; Newton et al., 2013; Öllerer et al., 2019). All of these factors are likely to have an effect on tick survival and tick-borne pathogen transmission.

Besides the obvious effects on vegetation, using livestock for land management can create competition with wild herbivore species and can lead to lower body condition in deer (Jenks and Leslie, 2003; Kuiters et al., 2005; Weiss et al., 2022). Close proximity between animals can also lead to disease transmission; many pathogens, including the agents of bovine tuberculosis, brucellosis or foot and mouth diseases can be transmitted between wild and domestic ungulates (Crispell et al., 2020; Mugabi and Duffy, 2023; Rayl et al., 2019; Small et al., 2002). In terms of vector-borne diseases, conservation grazing has recently been linked to the geographical spread of *Haemaphysalis punctata* ticks across an area in the south of England (Medlock et al., 2018).

In Europe, the most common tick species of public and veterinary health importance is the sheep tick, *Ixodes ricinus.* These ticks rely on vertebrate hosts for blood meals at each life stage (larvae, nymphs and adults). Larval and nymphal *I. ricinus* are commonly associated with small- and medium-sized vertebrates but can also feed on large vertebrate hosts (Kahl and Gray, 2023). For adult female *I. ricinus,* while they generally feed on larger mammals such as deer (in the UK roe deer [*Capreolus capreolus],* red deer *[Cervus elaphus]* or fallow deer *[Dama dama]*), which are known to drive tick densities (Gandy et al., 2022b, 2021; Gilbert et al., 2012), they are also frequently recorded on medium-size mammals such as squirrels (*Sciurus* spp.), hedgehogs (*Erinaceus europaeus*) and hares (*Lepus europaeus)* (Kahl and Gray, 2023). Livestock (cattle [*Bos taurus*] and sheep [*Ovis aries*]) can also feed a large number of ticks (Gilbert et al., 2017; L'Hostis et al., 1994), but few studies have investigated how they may affect tick densities. Research in the Netherlands using paired experiments to understand how cattle and sheep may affect tick-borne diseases found that grazing could sometimes reduce the density of ticks (Gassner et al., 2008; Sprong et al., 2020). This reduction in ticks has been hypothesised to be due to a lack of understory in grazed woodlands and by the reduction of small mammals overall, which provide blood meals for immature tick stages (van Wieren and Hofmeester, 2016).

Ixodes ricinus is a known vector of many pathogens including tick-borne encephalitis virus, *Borrelia burgdorferi* sensu lato (s.l.) (causing Lyme borreliosis) and *Anaplasma phagocytophilum* (causing anaplasmosis). *Anaplasma phagocytophilum* can be maintained and transmitted by small mammals, birds and some species of deer while horses (*Equus caballus*) and cattle are also suspected to transmit *A. phagocytophilum* (Matei et al., 2019; Stuen et al., 2013). *Borrelia burgdorferi* s.l. is a complex of bacteria comprising multiple genospecies, some of which are known to occur in the UK: *B. garinii* and *B. valaisiana*, which are primarily transmitted by birds (Hanincová et al., 2003), *Borrelia afzelii* which is transmitted by small mammals (van Duijvendijk et al., 2015) and *Borrelia burgdorferi* s.s., a more generalist species that has been found in a variety of vertebrates, including grey squirrels (*Sciurus carolinensis*) and bank voles (*Myodes glareolus*) (Kurtenbach et al., 1998). However, *B. burgdorferi* s.l. cannot be transmitted by deer or cattle (Jaenson and Tälleklint, 1992; Kraiczy, 2016, 2016; Richter and Matuschka, 2010), and some studies have shown that high densities of deer can lead to a decrease in *B. burgdorferi* s.l. prevalence in ticks, by diverting ticks from feeding on transmission hosts (Gandy et al., 2022b, 2021; Vourc'h et al., 2016).

To our knowledge, no study has examined the effect of conservation grazing on tick-borne disease hazard in British woodlands and, with new incentives encouraging farmers and landowners to take rewilding actions across the country, it is important to investigate how conservation grazing could affect tick-borne pathogen hazard (density of infected ticks), the combination between pathogen prevalence and tick density. Whilst conservation grazing has been used for centuries in some locations, it has been used more widely in the last two decades and it is difficult to assess how it could affect tick-borne disease hazard in the long term. To investigate this, this study used the New Forest National Park in southern England as an example of a rewilded landscape. This vast area of 60,000 hectares (ha) has been home to free-roaming livestock (ponies, donkeys [*Equus asinus*], cattle, pigs [*Sus scrofa domesticus*]) since the 11th century (Putman, 1986) and some of the woodlands (about 8300 ha) have been enclosed to protect parts of the forest from livestock (but not from deer) to support timber production since the 17th century. These fenced areas, called inclosures (a term we will retain for UK forestry relevance), persist to this day and provide an opportunity to test our hypotheses on the role of large domestic ungulates on tick-borne disease hazard.

Using a paired experiment, we assessed the effect of long-term grazing on ground vegetation height, *I. ricinus* density and prevalence of two pathogens: *B. burgdorferi* s.l. and *A. phagocytophilum* within a woodland ecosystem. As high-intensity grazing is likely to impact ground vegetation, we predicted that ground vegetation should be lower, with an increase in bare soil cover in areas grazed by livestock. While we did not specifically investigate the effect of livestock grazing on small mammal abundance, we hypothesised that small mammal abundance should be lower where livestock graze (Gassner et al., 2008; Putman et al., 1989; van Wieren and Hofmeester, 2016). Therefore, we predicted that tick density should be lower in grazed plots, via potential impacts on small mammals, which are important larval hosts, and vegetation cover. As we expected grazed woodland to have fewer rodents, ticks collected from grazed plots should have a lower prevalence of *B. burgdorferi* s.l., as they would be more likely to feed on cattle and horses, which are not known to transmit the bacteria (Kraiczy, 2016). However, the density of deer in woodland excluded from conservation grazing would potentially cause a confounding impact to this dilution effect, as deer also contribute to the reduction of *B. burgdorferi* s.l. infection rates in ticks. Regarding *A. phagocytophilum,* cattle, deer and livestock are suspected to be transmission hosts and thus, we were not expecting differences for *A. phagocytophilum* prevalence between plots. Finally, the impact of livestock grazing on the density of infected nymphs (DIN) will depend on impacts on tick density and pathogen prevalence.

2. Materials and methods

2.1. Location

The study took place in the New Forest National Park in southern England (50.876 N, 1.631 W) (Fig. 1). Established as a royal forest in the 11th century, it has retained its boundary since the late 13th century (Putman, 1986). Due to commoners' rights, livestock owners have been grazing their animals freely in the forest since the 11th century. New Forest ponies roam freely year-round whilst most of the cattle are kept inside in winter months (although some roam all-year-round) and pigs are only released in the forest in the autumn. The number of livestock roaming the forest increased from ~400 ponies and ~900 cattle in the 1930s to about 4900 ponies and 2800 cattle in 2023 (Verderers of the New Forest, 2023) (Table S1 for stock numbers per year). The New Forest comprises about ~9500 ha of heathland, ~4500 ha of grassland, ~8000 ha of deciduous woodlands and ~5000 ha of coniferous

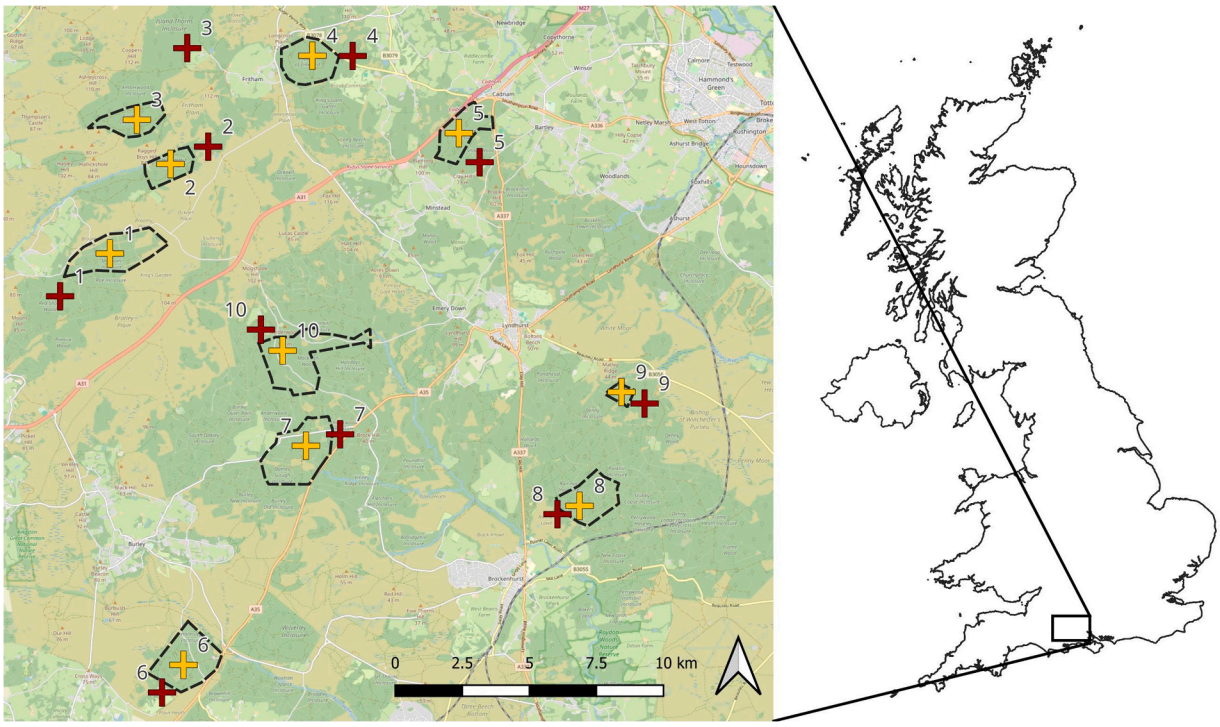

Fig. 1. Map of the survey locations in southern England. Crosses in orange represent inclosures and crosses in red represent adjacent woodland sites outside inclosures. Surveyed inclosures are represented in the map as polygons (see Table 6 for site coordinates).

woodland (Putman, 1986). Wild herbivores are also present with ~1300 fallow deer, 350–400 roe deer, 100 sika deer (*Cervus nippon*), 90 red deer and a few muntjac deer (*Muntiacus reevesi*) (New Forest National Park, 2023). As of 1980, there were 8300 ha of enclosed land for sylviculture which deer could access but were fenced against livestock.

2.2. Survey design and tick collection

For this study, 10 separate paired woodlands were sampled both at a location within the fenced inclosure and at an adjacent uninclosed (i.e., unfenced) ancient semi-natural woodland where cattle and ponies could graze. All 20 woodland sites were open to access for deer. Tick surveys were conducted three times at each site, between 0930 h and 1600 h on a dry day during one week in June 2021, 2022, and 2023. Ticks were collected using a standard flagging method (Falco and Fish, 1992), which involved dragging a 1 m x 1 m cotton cloth over vegetation. Surveys were done at least 50 m away from the fence on either side to avoid edge effects. For each site, 10 m long linear transects were conducted and repeated 20 times per location to obtain tick density. If the number of nymphs collected after the 20 transects was under 50, dragging was continued until at least 50 questing nymphs from each site were collected (where possible) for pathogen screening. However, these "extra" nymphs were not included in density estimates. Ticks were counted and collected using tweezers at the end of each transect and stored at -80 °C until identification and testing. For each transect, various vegetation variables were recorded including dominant ground vegetation type (bracken: *Pteridium aquilinum*, grass, Ericaceous/*Vaccinium* species, leaf litter), and vegetation height at the beginning (0 m), middle (5 m) and end (10 m) of the transect. To ensure the inclosures were properly fenced against livestock, we carefully inspected the area for pony or cattle dung during the survey inside inclosures. Information on the date of survey, time of survey, temperature and woodland type was recorded for each site.

2.3. Detection of *B. burgdorferi* s.l. and *A. phagocytophilum*

Ticks were morphologically identified (Estrada-Peña, 2018; Hillyard, 1996) and up to 50 questing *I. ricinus* nymphs were tested per site and per year based on the results of a power analysis using the average prevalence for *B. burgdorferi* s.l. and *A. phagocytophilum*, based on published studies that compared infection rates with and without livestock grazing (Gassner et al., 2008; Sprong et al., 2020). The DNA from questing nymphs were individually extracted using the ammonia extraction method previously described (Hansford et al., 2015). The DNA extracts were tested for the presence of *B. burgdorferi* s.l. (Parola et al., 2011) and *A. phagocytophilum* (Courtney et al., 2004) using PCR assays previously published (Table 1). The qPCRs were implemented using Taq Man™ Fast Universal master mix (Applied Biosystems) in a QuantStudio 7 Flex real-time PCR system (Applied Biosystems). Each reaction of 20 µL contained 10 µL of master mix, 1 µL of primers/probe mix, 4 µL of RNase free water and 5 µL of DNA extract using a program consisting of 20 s at 95 °C, followed by 40 cycles of 3 s at 95 °C and 30 s at 60 °C. One positive DNA control and one negative control were included for every plate.

A number of *A. phagocytophilum msp2* gene qPCR-positive samples were further analysed by amplifying a partial fragment of the *groEL* gene using a previously published heminested PCR assay (Apaa et al., 2023). Briefly, PCR reactions were performed using a 25 µL total volume for both rounds. For the first round, the master mix included 12.5 µL 2x iTaq universal SYBR green master mix (BIO-RAD), 1 µL of both forward (*groEL* 569) and reverse (*groEL* 1193) primers, 8.5 µL nuclease free water and 2 µL template DNA. The second round used the same mix, replacing the reverse primer with *groEL* 1142. Thermal cycling for both rounds consisted of 94°C for 5 min, followed by 40 cycles (94°C for 30 s, 56°C – round 1 or 60°C – round 2 for 45 s, 72°C for 1 min) and a final extension at 72°C for 3 min. PCR products (~600 bp) were separated on a 1.5 % agarose gel and Sanger sequenced to determine the ecotype (Jahfari et al., 2014).

To determine the *B. burgdorferi* s.l. genospecies, positive samples were sequenced using the 5S-23S rRNA intergenic spacer region

Table 1
Primers and probes used for the detection of B. burgdorferi s.l. and A. phagocytophilum in questing I. ricinus nymphs.

Pathogen	Target gene	Primer name	Nucleotide sequence (5′ → 3′)
Borrelia burgdorferi s.l.	16S rRNA	Primer forward	AGCCTTTAAAGCTTCGCTTGTAG
		Primer reverse	GCCTCCCGTAGGAGTCTGG′
		Probe	6FAM-CCGGCCTGAGAGGGTGAACGG-BHQ1
Anaplasma phagocytophilum	msp2	Primer forward	ATGGAAGGTAGTGTTGGTTATGGTATT
		Primer reverse	TTGGTCTTGAAGCGCTCGTA
		Probe	TGGTGCCAGGGTTGAGCTTGAGATTG
Anaplasma phagocytophilum ecotyping	*groEL*	*groEL* 569	ATGGTATGCAGTTTGATCGC
		Round 1 - *groEL* 1193	TCTACTCTGTCTTTGCGTTC
		Round 2 – *groEL* 1142	TTGAGTACAGCAACACCACCGGAA
Borrelia burgdorferi s.l. sequencing	5S-23S rRNA intergenic spacer region	Forward	GAG TTC GCG GGA GAG TAG GTT ATT GCC
		Reverse	TCA GGG TAC TTA GAT GGT TCA CTT CC

(*) Primer used in both rounds 1 and 2 for *A. phagocytophilum* ecotyping.

(Alekseev et al., 2001). A 50 µL reaction was prepared containing 5 µL 10 × PCR reaction buffer, 1 µL 10 mM dNTPs, 1.5 µL 50 mM $MgCl_2$, 2 µL of each primer from a 10 µM stock (Table 1), 0.2 µL Platinum Taq DNA polymerase (Invitrogen) and 33.3 µL PCR grade H_2O. PCR reactions were carried out under the following cycling conditions: 5 min at 94°C, followed by 10 cycles of 94°C for 20 s, 70°C for 30 s (lowering by 1°C each cycle) and 72°C for 30 s, then 40 cycles of 94°C for 20 s, 60°C for 30 s and 72°C for 30 s, with a final extension of 72°C for 7 min. PCR products were sent to the UK Health Security Agency Genomic Service and Development Unit (Colindale, London) for purification and bidirectional Sanger sequencing. The chromatograms of the sequences were visually inspected and trimmed using SnapGene® Viewer (Dotmatics, Massachusetts, USA). Forward and reverse sequences were aligned in MEGA 11 using the MUSCLE algorithm to generate a consensus sequence. *Borrelia burgdorferi* s.l. genospecies was determined by inputting consensus sequences into BLAST (NCBI). Sequences have been uploaded to GenBank (GenBank accession numbers: PV848209-PV848346)

2.4. Statistical analysis

All statistical analyses were performed in R (version 4.2.2) using the *glmmTMB, MuMIn* and *car* packages (Barton, 2009; Brooks et al., 2017; Fox and Weisbert, 2019). Generalised linear mixed effect models (GLMMs) were used to understand how grazing by livestock might impact vegetation height, tick densities, nymphal infection prevalence (NIP) and density of infected nymphs. For each model, we tested collinearity between explanatory variables using generalised variance inflation factor (GVIF) (Fox and Monette, 1992) and we tested for overdispersion and zero-inflation when appropriate using the *DHARMa* package (Hartig, 2022). Model selection was done based on AICc (Brewer et al., 2016) using the *dredge* function from the *MuMIn* package and we selected the model with the lowest AICc, to balance explanatory power while avoiding overfitting. We performed model diagnostics using the *DHARMa* package. Multi-level categorical variables were analysed using post-hoc Tukey tests to identify significant differences between categories.

To investigate how grazing might affect vegetation height, we used a hurdle negative binomial GLMM with the response variable being the average ground vegetation height at the transect level rounded to the nearest integer. The full model included site type (inside inclosures vs outside inclosures), the dominant ground vegetation at the transect level (bracken, grass, bracken/grass, Ericaceous/*Vaccinium* species, leaf litter), woodland type (coniferous, deciduous and mixed woodland), year (2021, 2022, 2023) and the interaction between site type and the dominant ground vegetation. Site name was added as a random effect to account for variability between sampling locations.

To understand how grazing by livestock might impact tick densities, we used a negative binomial GLMM with the response variable being the number of questing *I. ricinus* nymphs per transect. The full model included site type (inside inclosures vs outside inclosures), as well as other variables, which we know can affect questing activity and blanket dragging efficiency, that were year (2021–2023), dominant ground vegetation at transect level (bracken, grass, bracken/grass, Ericaceous/*Vaccinium* species, leaf litter), woodland type (coniferous, deciduous and mixed woodlands), average ground vegetation height per transect and temperature in its quadratic form as explanatory variables. Site name was added as a random effect.

To investigate how grazing can affect NIP, we ran four binomial GLMMs with the response variable being the proportion of nymphs infected (number infected vs number uninfected) with *B. burgdorferi* s.l. (model 1), *B. afzelii* (model 2), *B. garinii* and *B. valaisiana* (model 3) and *A. phagocytophilum* (model 4) at the site/visit level (one estimate per site per year). We chose to have one model focusing on *B. afzelii* as this genospecies is transmitted by rodents (van Duijvendijk et al., 2015) and one model combining infection prevalence with *B. garinii* and *B. valaisiana*, as these are mainly transmitted by birds (Hanincová et al., 2003), to understand how host community composition might be different inside and outside inclosures. Fixed covariates included site type (inside inclosures vs outside inclosures), year (2021, 2022, 2023), woodland type (coniferous, deciduous and mixed woodland) and the prevalence of the other pathogen to investigate potential competition within the tick (i.e. *B. burgdorferi* s.l. or *A. phagocytophilum*) (Gandy et al., 2022a). Site name was added as a random effect.

In a similar way, we ran four zero-inflated negative binomial GLMMs (for *B. burgdorferi* s.l., *B. afzelii, B. garinii* and *B. valaisiana* and *A. phagocytophilum*) to understand how grazing affected the DIN. The response variable was the density of infected nymphs (one estimate per site per year) and we used an offset for the area surveyed (Zuur et al., 2009). The full model included site type as well as other variables we know can affect tick questing activity and blanket dragging efficiency: year, temperature, woodland type, average ground vegetation height (at the site level) and DIN for the other pathogen, to account for potential competition within the tick. Site was added as a random effect.

3. Results

3.1. Impacts of livestock grazing on vegetation

3.1.1. Vegetation data summary

For ground vegetation, the proportion of transects that had bracken or Ericaceous/*Vaccinium* as the dominant ground vegetation was similar between inclosures and outside inclosures (bracken: 9.2 % in inclosures vs 8.5 % outside inclosures; Ericaceous/*Vaccinium*: 0.8 % vs 0.7 %, Fig. 2). In inclosures, the dominant ground vegetation was bracken/grass (46.7 % of transects) whilst bracken/grass was the dominant ground vegetation for 10.7 % of transects where livestock grazed only. The proportion of transects with grass as the dominant vegetation was higher where livestock grazed (30.2 % vs 11.7 %) and leaf litter as the dominant vegetation (i.e., no ground vegetation) represented 50 % of

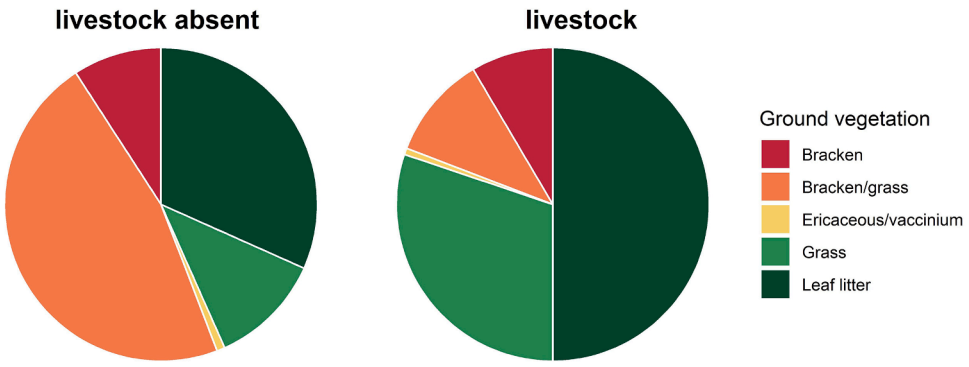

Fig. 2. Pie chart representing the proportion of transect that had bracken (red), bracken/grass (orange), Ericaceous/*Vaccinium* (yellow), Grass (light green), leaf litter (dark green) as the dominant ground vegetation in the absence (inclosures) and presence (outside inclosures) of livestock.

transects where livestock grazed compared to 31.7 % of transects in inclosures (livestock absent). Inclosures consisted of deciduous (57 %), coniferous (30 %) and mixed (23 %) woodlands whilst adjacent sites with livestock were only deciduous woodlands (as no coniferous forest was planted outside inclosures).

3.1.2. *Model predictions for vegetation type and height*

The selected model investigating how livestock grazing may impact ground vegetation height included year and the interaction between site (outside inclosures vs inside inclosures) and dominant ground vegetation (Table 2). Comparison for vegetation height in different vegetation types and site type were obtained using post-hoc Tukey tests. When the ground vegetation was a mix between bracken and grass or grass, ground vegetation was significantly lower outside inclosures where livestock grazed (bracken/grass: 24.2 cm [95 %CI: 19.8–29.6], grass: 7.9 cm [95 %CI: 6.7–9.5]) compared to inside inclosures (bracken/grass: 40.8 cm [95 %CI: 34.8–47.9], p<0.001, grass: 18.4 cm [95 %CI: 15.0–22.4], p<0.001). There was no significant difference between vegetation height inside and outside inclosures when the dominant ground vegetation was bracken (predicted vegetation height where livestock grazed: 33.9 cm [95 %CI: 27.2–42.1] vs 35.3 cm [95 %CI: 28.3–44.0] where livestock did not graze, p = 1.0), Ericaceous/*Vaccinium* (28.4 cm [95 %CI: 16.4–49.2] vs 25.9 cm [95 %CI: 15.7–42.8], p = 1.0) or leaf litter (7.94 cm [95 %CI: 6.67–9.45] vs 13.8 cm [95 %CI: 10.6–17.9], p=0.82).

Regarding the zero-inflated part of the model, the probability of having no ground vegetation (i.e., 0 cm) was significantly higher outside inclosures where livestock graze compared to inside inclosures (Table 2).

Table 2
Outputs from the selected generalized linear mixed effect model explaining the effects of grazing, ground vegetation and year on ground vegetation height.

	Estimate	Std. Error	z value	Pr (>|z|)	ΔAICc[a]
Conditional model:					
(Intercept)	3.74	0.11	33.4	< 0.001	
Type: Inclosures vs outside	0.04	0.12	0.34	0.73	
Ground vegetation (Baseline: Bracken)					
Bracken/grass	-0.34	0.11	-3.18	0.001	
Ericaceous/*Vaccinium*	-0.17	0.28	-0.62	0.54	
Grass	-1.45	0.10	-14.41	<0.001	
Leaf litter	-2.14	0.74	-2.87	0.004	
Year (Baseline: 2021)					48.4
2022	-0.34	0.05	-6.50	<0.001	
2023	-0.33	0.05	-6.16	<0.001	
Type * Ground vegetation (Baseline: ASNW) 22.4					
Inclosure & Bracken/grass	0.48	0.14	3.41	<0.001	
Inclosure & Ericaceous/*Vaccinium*	-0.13	0.38	-0.35	0.72	
Inclosure & Grass	0.80	0.15	5.25	<0.001	
Inclosure & Leaf litter	1.19	0.75	1.59	0.11	
Zero-inflation model:					
(Intercept)	-4.49	0.80	-5.62	<0.001	
Type: Inclosures vs outside	-2.47	0.47	-5.23	<0.001	23.2
Year (Baseline: 2021)					16.9
2022	1.72	0.41	4.19	<0.001	
2023	1.14	0.42	2.73	0.006	
Ground vegetation (Baseline: Bracken) 536.1					
Bracken/grass	-0.06	0.95	-0.06	0.95	
Ericaceous/*Vaccinium*	-13.04	2131.48	-0.006	0.99	
Grass	1.03	0.77	1.33	0.18	
Leaf litter	8.03	0.86	9.34	<0.001	

[a] The ΔAICc refers to the effect of removing the variable in the given row on the AICc of the best model.

3.2. *Impacts of livestock grazing on the density of questing ticks, nymphal infection prevalence and the density of infected nymphs*

3.2.1. *Tick data summary*

Between 2021 and 2023, 2906 questing *I. ricinus* were collected (2522 nymphs, 184 adult males and 200 adult females) along transects with an average of 21.0 ± 28.3 questing nymphs/100 m², 1.5 ± 4.5 adult males/100 m² and 1.7 ± 4.4 adult females/100 m² (Table 3). Nymph density was 23.3 ± 32.3 nymphs /100 m² inside inclosures and 18.8 ± 23.4 nymphs/100 m² outside inclosures (Fig. 3A).

Between 2021 and 2023, 2974 nymphs were tested for pathogens, with an overall prevalence of 6.3 % [95 %CI: 5.4–7.1] (n=186 positive samples) for *B. burgdorferi* s.l. (Table 3). In terms of genospecies, 43.0 % (80/186) of positive samples were *B. garinii*, 21.5 % (40/186) *B. valaisiana,* and 9.7 % (18/186) *B. afzelii.* Overall, 25.8 % (48/186) of positive samples could not be attributed to a genospecies. *Borrelia burgdorferi* s.l. infection prevalence outside the inclosures was 7.2 % [95 %CI: 5.9–8.5] (107/1490) with 42 % (45/107) of positive samples being *B. garinii,* 24.4 % (25/107) *B. valaisiana* and 13.1 % (14/107) *B. afzelii.* Inside enclosures, 5.3 % of nymphs were infected [95 %CI: 4.2–6.5] (79/1484) with 44.3 % (35/79) of positive sampled attributed to *B. garinii,* 19.8 % (15/79) to *B. valaisiana* and 5.1 % (4/79) to *B. afzelii* (Fig. 3B & D).

For *A. phagocytophilum*, we found an average prevalence of 3.1 % (92/2974) [95 %CI: 2.5–3.7] (Table 3). Infection prevalence outside the inclosures was 2.5 % [95 %CI: 1.7–3.3] (37/1490) and 3.7 % [95 %CI: 2.7–4.7] (55/1484) inside inclosures (Fig. 3B). A subset of samples (n = 22) could be attributed to an ecotype; 91 % were ecotype I (20/22) and 9 % were ecotype II (2/22) (Table S3). For ecotype I, 12 samples came from inside inclosures and 8 from outside and, for ecotype II, one sample came from inside an inclosure and one from outside

On average the density of nymphs infected with *B. burgdorferi* s.l. was 1.3 ± 1.6/100 m² compared to 0.7 ± 0.9/100 m² for *A. phagocytophilum* (Table 3). The density of nymphs infected inside inclosures was 1.3 ±

Table 3
Summary of the different locations surveyed during the study with the average density of *I. ricinus* nymphs (DON) per 100 m² (± SD), nymphal infection prevalence (NIP,) (number positive/number tested) and density of infected *I. ricinus* nymphs (DIN) per 100 m² (± SD) for *B. burgdorferi* s.l. and *A. phagocytophilum*. Incl: Inside inclosures; ASNW: ancient semi natural woodland (outside inclosures).

Location	Type	Coordinates	DON	NIP *B. burgdorferi*	DIN *B. burgdorferi*	NIP *A. phago-cytophilum*	DIN *A. phago-cytophilum*
1- Redshoot	ASNW	50.882917, -1.726767	17.2±18.9	4 % (6/150)	0.6±0.2	2.7 % (4/150)	0.5±0.5
1- Redshoot	Incl	50.886772, -1.721184	31.0±36.3	4 % (6/149)	0.9±0.5	2 % (3/149)	0.2±0.4
2- Ocknell	ASNW	50.910403, -1.677671	24.3±36.2	11.3 % (17/150)	3.1±2.4	3.3 % (5/150)	1.0±1.2
2- Ocknell	Incl	50.907869, -1.681488	28.8±50.3	6 % (9/150)	1.4±0.9	6.7 % (10/150)	2.5±2.9
3- Eyeworth	ASNW	50.931755, -1.679578	7.8±9.3	6.8 % (10/147)	0.6±0.4	2.7 % (4/147)	0.2±0.1
3- Eyeworth	Incl	50.919339, -1.695664	25.0±24.6	2 % (3/150)	0.7±0.9	4 % (6/150)	0.7±0.7
4- Brook	ASNW	50.927831, -1.624771	27.2±19.5	1.3 % (2/150)	0.4±0.6	2.7 % (4/150)	0.7±0.2
4- Brook	Incl	50.93242, -1.645774	24.2±21.7	4 % (6/150)	1.1±0.8	0.7 % (1/150)	0.2±0.3
5- Shave	ASNW	50.906451, -1.591735	15.2±16.7	8.2 % (12/146)	1.1±0.7	1.4 % (2/146)	0.3±0.5
5- Shave	Incl	50.907242, -1.593572	12.0±12.9	7.3 % (11/150)	0.7±0.6	1.3 % (2/150)	0.2±0.2
6- Holmsley	ASNW	50.796102, -1.689868	32.0±34.4	8.8 % (13/148)	2.7±1.3	3.4 % (5/148)	0.9±0.4
6- Holmsley	Incl	50.796102, -1.689868	33.7±32.4	11.2 % (16/143)	4.1±4.0	4.9 % (7/143)	1.5±0.7
7- Vinney	ASNW	50.84919, -1.63592	18.7±24.5	7.3 % (11/150)	1.1±0.4	0 % (0/150)	0
7- Vinney	Incl	50.848664, -1.636357	14.0±21.2	4.7 % (7/150)	0.6±0.5	6.0 % (9/150)	0.8±0.7
8- Pignal	ASNW	50.832911, -1.557696	12.2±11.1	11.5 % (18/156)	1.6±1.2	3.8 % (6/156)	0.4±0.3
8- Pignal	Incl	50.832837, -1.554271	13.0±18.5	5.3 % (8/150)	0.7±0.7	4.7 % (7/150)	0.7±0.5
9- Denny	ASNW	50.855206, -1.528368	13.5±17.9	7 % (10/143)	0.4±0.4	3.5 % (5/143)	0.4±0.2
9- Denny	Incl	50.860558, -1.528034	28.8±51.6	6.3 % (9/143)	2.4±3.3	2.8 % (4/143)	1.2±1.7
10- Bolderwood	ASNW	50.869226, -1.656211	19.7±20.8	5.3 % (8/150)	1.0±0.8	1.3 % (2/150)	0.3±0.5
10- Bolderwood	Incl	50.869621, -1.657094	22.3±21.7	2.7 % (4/149)	0.5±0.8	4 % (6/149)	1.0±1.0

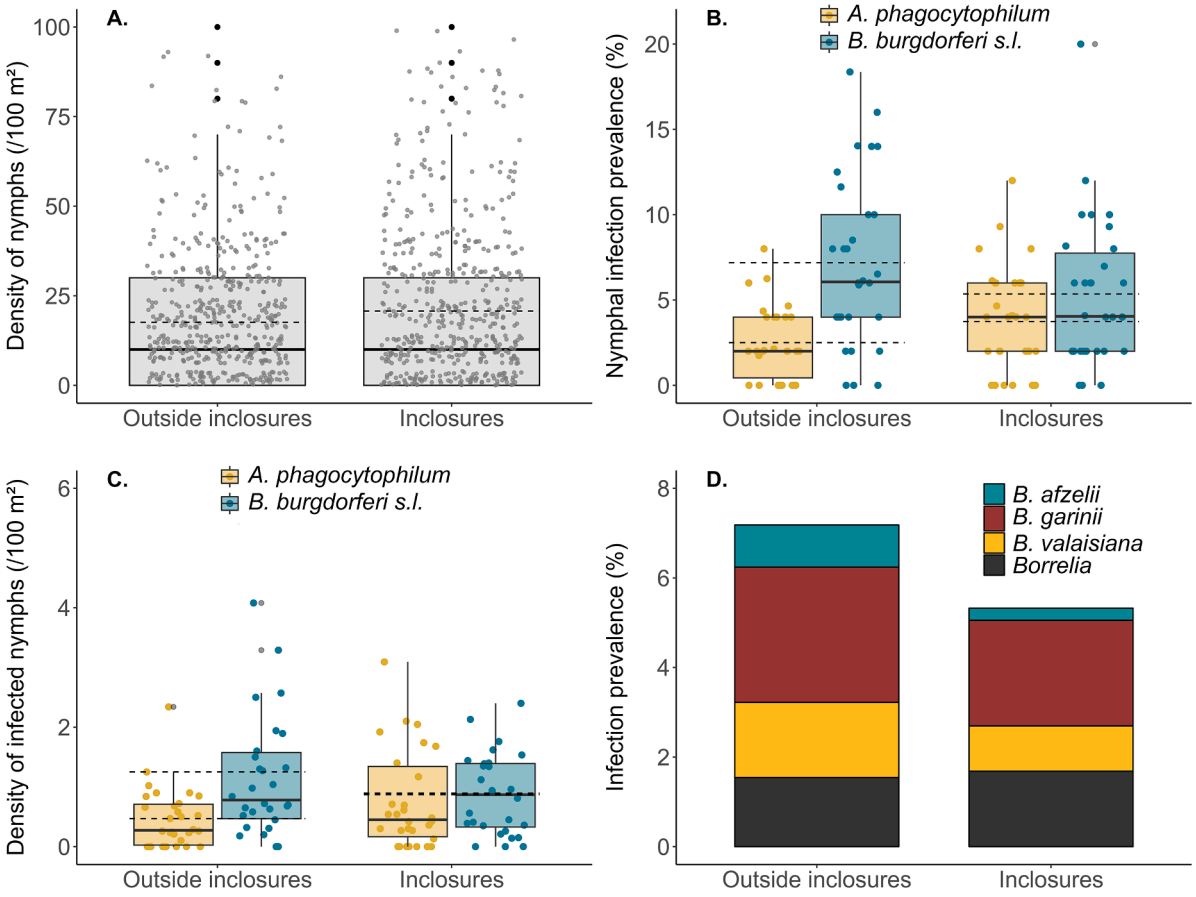

Fig. 3. Boxplots showing raw data for (A.) the density of questing *I. ricinus* nymphs (/100 m²), (B.) nymphal infection prevalence for *B. burgdorferi* s.l. (blue) and *A. phagocytophilum* (orange), (C.) the density of infected *I. ricinus* nymphs for *B. burgdorferi* s.l. (blue) and *A. phagocytophilum* (orange) and (D.) the prevalence of the different *B. burgdorferi* s.l. genospecies outside and inside inclosures.

1.8/100 m² for *B. burgdorferi* s.l. and 0.9 ± 1.2/100 m² for *A. phagocytophilum*. Outside inclosures, the density of infected nymphs was 1.3 ± 1.3/100 m² for *B. burgdorferi* s.l. and 0.5 ± 0.5/100 m² for *A. phagocytophilum* (Fig. 3C).

3.2.2. Impacts of livestock grazing on the density of nymphs (DON) – model predictions

The selected model to explain variation in DON included site type, year, dominant ground vegetation, vegetation height and temperature in its quadratic form (Table 4). Nymph density was significantly higher

Table 4
Outputs from the selected generalised linear mixed effect model explaining the effects of grazing, ground vegetation, temperature, vegetation height and year on the density of questing *I. ricinus* nymphs.

	Estimate	Std. Error	z value	Pr(>|z|)	ΔAICc[a]
(Intercept)	0.34	0.17	1.99	0.04	
Type: Inclosures vs outside	0.24	0.07	3.24	0.001	8.4
Ground vegetation (Baseline: Bracken)					9.2
Bracken/grass	0.39	0.14	2.76	0.01	
Ericaceous/*Vaccinium*	0.86	0.38	2.27	0.02	
Grass	0.09	0.15	0.58	0.56	
Leaf litter	0.02	0.15	0.15	0.88	
Year (Baseline: 2021)					118.3
2022	-0.23	0.10	2.25	0.02	
2023	0.66	0.10	6.40	<0.001	
Vegetation height	-0.25	0.05	-4.69	<0.001	20.2
Temperature	0.10	0.05	1.86	0.06	6.4
Temperature^2	-0.13	0.05	-2.90	0.004	6.2

[a] The ΔAICc refers to the effect of removing the variable in the given row on the AICc of the best model.

inside the inclosures (predicted DON: 19.3/100 m^2 [95 %CI: 12.2–31.9]) compared to outside, where livestock grazed (predicted DON: 15.2/100 m^2 [95 %CI: 9.5–25.4], p = 0.001). Nymph density also varied depending on year, the ground vegetation, vegetation height and temperature (Table 4, see Table S2 for Tukey tests results).

3.2.3. *Impacts of livestock grazing on nymphal infection prevalence (NIP) – model predictions*

The best model to explain the variation in NIP for *B. burgdorferi* s.l. was the null model and it seemed that NIP for *B. burgdorferi* s.l. did not vary as a function of site type (i.e., grazing) (Table S3). For the model focusing on the genospecies transmitted by rodents, *B. afzelii*, the best model included site type and prevalence of the bird-transmitted genospecies (*B. garinii* and *B. valaisiana*). The prevalence of *B. afzelii* was positively correlated with prevalence of the bird genospecies (p = 0.007) and was slightly lower inside inclosures (0.3 %, 95 % CI: 0.1–0.8) compared to outside inclosures (0.75 %, 95 % CI: 0.4–1.6, p = 0.09) where livestock grazed (Table 5).

For the model focusing on the genospecies transmitted by birds (*B. garinii* + *B. valaisiana*), the best model included the prevalence of the rodent transmitted genospecies (*B. afzelii*) only (Table S3).

The selected model to explain the variation in NIP for *A. phagocytophilum* included woodland type only and NIP was not affected by site type (i.e., grazing) (Table S3).

3.2.4. *Impacts of livestock grazing on the density of infected nymphs (DIN) – model predictions*

The best model to explain the variation in DIN for *B. burgdorferi* s.l. included year, ground vegetation height and DIN for *A. phagocytophilum* and DIN did not seem to vary as a function of site type (i.e., grazing) (Table S4). The best model to explain the variation in DIN for *B. afzelii* included temperature, DIN for *A. phagocytophilum* and DIN for the bird associated genospecies (Table S4).

The best model to explain the variation in DIN for the bird genospecies (*B. garinii* and *B. valaisiana*) included site type, ground vegetation height and DIN for *B. afzelii* (Table 6). DIN for the bird genospecies was significantly higher inside inclosures (0.1/100 m^2, 95 % CI: 0.06–0.3) compared to outside inclosures (0.06/100 m^2, 95 % CI: 0.03–0.1, p = 0.007).

The best model to explain the variation in the DIN for *A. phagocytophilum* included habitat, year and DIN for *B. burgdorferi* s.l., so DIN did not seem to vary as a function of site type (i.e., grazing) (Table S4).

4. Discussion

This study aimed to investigate the impact of long-term naturalistic grazing by horses and cattle on the dynamics of infection with tick-borne pathogens through a paired experiment conducted in the New Forest in southern England. We found that grazing by livestock led to shorter ground vegetation (when the vegetation was grass and grass/bracken) and an increase in bare soil cover. We also found that grazing by livestock led to a decrease in the density of questing *I. ricinus* nymphs. In terms of pathogens, we focused on *B. burgdorferi* s.l., causing Lyme borreliosis in humans and livestock, and *A. phagocytophilum*, causing human granulocytic anaplasmosis and tick-borne fever in livestock. Although nymphal infection prevalence with *B. burgdorferi* s.l. was higher outside the inclosures (7.2 % outside vs 5.3 % inside) and higher for *A. phagocytophilum* inside the inclosures (2.5 % outside vs 3.7 % inside), these differences were not statistically significant. Our assay did not differentiate between *B. burgdorferi* s.l. and *Borrelia miyamotoi*, so some of our positive samples that could not be attributed to a genospecies could have been *B. miyamotoi.* However, a study conducted in England and Wales tested over 4000 nymphs and found that only 0.2 % were infected with *B. miyamotoi,* so prevalence is presumed to be very low (Cull et al., 2021).

We did not find any significant differences in nymphal infection prevalence for the genospecies transmitted by rodents (*B. afzelii*) and birds (*B. garinii* and *B. valaisiana*) between our treatments. The density of infected nymphs with *B. burgdorferi* s.l., *B. afzelii,* and *A. phagocytophilum* were not significantly different inside compared to outside inclosures. However, the density of infection nymphs with *B. garinii* and *B. valaisiana* was significantly higher inside inclosures. Detection of ecotypes I and II in infected ticks further corroborates previous studies identifying these ecotypes in *I. ricinus* in the UK (Apaa et al., 2023; Gandy et al., 2022a; Medlock et al., 2024). In line with other studies conducted in England, *B. garinii* was the dominant genospecies followed by *B. valaisiana* and *B. afzelii* (Cull et al., 2021; Medlock et al., 2022)

This study was conducted in the New Forest in southern England, the land and boundaries of which have remained intact for nine centuries, along with the historical presence of free-roaming livestock on the commons (Putman, 1986). In terms of tick-borne diseases, the New Forest is a hotspot for Lyme borreliosis, with an incidence up to 10 times

Table 5
Outputs from the selected generalised linear mixed effect model explaining the effects of grazing and prevalence of the bird genospecies on nymphal infection prevalence with *B. afzelii*.

	Estimate	Std. Error	z value	Pr(>|z|)	ΔAICc[a]
(Intercept)	-5.74	0.58	-9.94	<0.001	
Type: Inclosures vs outside	-0.98	0.59	-1.66	0.09	0.51
Prevalence *B. garinii and B. valaisiana*	0.18	0.07	2.70	0.007	4.82

[a] The ΔAICc refers to the effect of removing the variable in the given row on the AICc of the best model.

Table 6
Outputs from the selected generalised linear mixed effect model explaining the effects of grazing, vegetation height and DIN for *B. afzelii* on the density of *I. ricinus* nymphs infected with *B. garinii* and *B. valaisiana*.

	Estimate	Std. Error	z value	Pr(>|z|)	ΔAICc[a]
(Intercept)	-4.53	0.19	-23.49	<0.001	
Type: Inclosures vs outside	0.75	0.28	2.69	0.007	4.1
Vegetation height	-0.03	0.01	-3.24	0.001	6.3
DIN *B. afzelii*	0.04	0.02	1.85	0.06	1.2

[a] The ΔAICc refers to the effect of removing the variable in the given row on the AICc of the best model.

the national average in postcodes located inside and around the area (Tulloch et al., 2019). Whilst this higher incidence might be due to an increased awareness in this area, it could also be due to a higher environmental risk for Lyme borreliosis.

In line with our predictions, grazing by ponies and cattle had a significant impact on ground vegetation. The ground vegetation inside inclosures was predominantly bracken and grass compared to bare soil (leaf litter) in adjacent woodlands where livestock grazed (Alexander et al., 2023; Newton et al., 2013). This suggests that grazing by livestock, especially ponies, can increase bare soil cover (Newton et al., 2013; Öllerer et al., 2019). When the dominant ground vegetation was bracken only, Ericaceous/*Vaccinium* or leaf litter, the average ground vegetation height was similar inside and outside inclosures, which is consistent with the feeding ecology of livestock as bracken can, for example, cause poisoning. Livestock in this area seemed to primarily graze on grass, which was significantly shorter outside inclosures, in accordance with the feeding ecology of livestock in the New Forest.

When investigating the effect of livestock grazing, we found that nymph density was significantly higher inside inclosures. Due to the taller ground vegetation and reduced bare soil cover, inclosures may provide higher humidity and adequate microclimate for tick survival (Estrada-Peña, 2001; Milne, 1950; Pfäffle et al., 2013; Williams and Ward, 2010). Several studies, including some in the New Forest, have suggested that livestock pressure leads to a decrease in the number of small mammals via their impacts on ground vegetation (Putman, 1986; Putman et al., 1989; Schieltz and Rubenstein, 2016). We can, therefore, expect a higher density of small mammals in inclosures and consequently an additional significant source of blood meals for immature *I. ricinus,* likely further explaining the higher density observed (Krawczyk et al., 2020; Ostfeld et al., 2006). However, we did not find any significant difference between nymphal infection prevalence or the density of infected nymphs with *B. afzelii* (transmitted by rodents) inside and outside inclosures. In addition, if deer were present in higher densities inside inclosures, we would expect to have a higher nymph density, as deer are known to drive tick densities (Gandy et al., 2022b, 2021; Gilbert et al., 2012). Further work should investigate impacts of grazing pressure on other mammal species and compare densities of small mammals and tick burdens between sites. It would also have been interesting to accurately estimate the abundance of livestock and deer at each of our study sites to explore these relationships further. The use of ivermectin as an acaricide is banned across the National Park so livestock would not have been treated against ectoparasites, and this would be an important consideration in similar studies conducted elsewhere.

Regarding pathogen prevalence, we predicted that prevalence for *B. burgdorferi* s.l. should be higher in inclosures as livestock, which are suspected to be accidental hosts (Kraiczy, 2016; Lin et al., 2020), would reduce infection prevalence by diverting ticks from feeding on transmission hosts. However, we did not find any significant effect of livestock on prevalence for *B. burgdorferi* s.l. or *B. garinii* and *B. valaisiana* and we found that prevalence for *B. afzelii* was higher, although not significantly, outside inclosures, which is in line with previous studies (Gassner et al., 2008; Sprong et al., 2020). One reason for this lack of differences could be that more deer may seek refuge inside inclosures to avoid competition with livestock and to seek out more densely-vegetated habitats to rest in (Kuiters et al., 2005; Weiss et al., 2022). If deer density is higher inside inclosures, it could lead to lower *B. burgdorferi* s.l. prevalence, as deer do not transmit the pathogen (Kraiczy, 2016; Lin et al., 2020). This could explain why observed prevalence was similar between treatments, as livestock would lead to a reduction of the pathogen outside inclosures and deer inside inclosures. However, we did not collect any data on deer abundance so we cannot investigate this relationship. If deer were congregating in smaller, sheltered areas, it could create an island effect. However, it is unlikely to be the case here as the smallest inclosure we used was 20 ha and most of the inclosures are well connected. Another limitation of this study is the assumption that livestock could not access the inclosures. However, some fences may have been damaged, potentially allowing cattle and ponies to enter and, while we did not observe any evidence of livestock (e.g. dung) inside the inclosures during the surveys, their presence cannot be completely ruled out, but their density is likely to be very low.

Regarding the prevalence of *A. phagocytophilum,* we did not expect to find differences as deer are transmission hosts and livestock are suspected to maintain and transmit the bacteria (Matei et al., 2019; Stuen et al., 2013). In line with our prediction and as supported by another study, we did not find any significant difference in prevalence inside as opposed to outside inclosures (Sprong et al., 2020). As previously explained, this could again be due to the fact that more deer may use inclosures to avoid competition with livestock and, as they are suspected to be transmission hosts for *A. phagocytophilum* (Matei et al., 2019; Stuen et al., 2013). A higher density could explain why prevalence was equal between treatments (Sprong et al., 2020). We could not use our ecotyping data to investigate this hypothesis further as only two positive samples were assigned to ecotype II, which is associated with roe deer (Jahfari et al., 2014). Recent research have found a positive association between sheep presence and prevalence for *A. phagocytophilum* (Gandy et al., 2022a; Medlock et al., 2024), showing that conservation grazing can lead to an increase in its prevalence.

We observed no significant differences in the density of infected nymphs for either *A. phagocytophilum, B. burgdorferi* s.l., or *B. afzelii*, consistent with findings from Sprong et al. (2020). However, we found that the density of infected nymphs with *B. garinii* and *B. valaisiana,* the dominant genospecies in the system, was higher inside inclosures, suggesting that grazing by livestock could lower disease hazard via their effects on nymph density or that higher abundance of birds are present inside inclosures, which should be investigated further. When we looked at the raw data for *A. phagocytophilum,* disease hazard was slightly higher inside inclosures as well. This implies that livestock grazing could lead to lower *A. phagocytophilum* hazard*,* probably through the negative effect of livestock on nymph density. One way to test this further would be to investigate whether deer actively graze in areas where livestock are absent and if they spend more time inside inclosures.

This study assessed tick-borne disease hazard in areas with livestock grazing but also in adjacent areas with no livestock. In this case, conservation grazing by ponies and cattle seemed to only have a small impact on disease hazard for genospecies that are transmitted by birds. Finally, the New Forest, due to its history, is a unique location in Europe. To understand if these results can be applied elsewhere, it would be interesting to include more locations where conservation grazing in woodland has been used, such as Ennerdale in the north of England, the Knepp estate in Sussex or Dartmoor national park in the southwest of England.

5. Conclusion

This study used a paired experiment to understand the impacts of horse and cattle naturalistic grazing on the hazard of two tick-borne pathogens within a woodland ecosystem. While grazing significantly impacted ground vegetation and reduced nymphal density, there was no observed effect of livestock on nymphal infection prevalence for *B. burgdorferi* s.l. and *A. phagocytophilum*. Regarding disease hazard, grazing by livestock had no impact on the density of nymphs infected with *A. phagocytophilum* or *B. burgdorferi* s.l. however, disease hazard for *B. garinii* and *B. valasiana* was lower where livestock grazed. These findings implies that conservation grazing by horses and cattle could have an impact on tick-borne disease hazard in this context. However, further research is needed to fully understand the underlying mechanisms.

Data statement

All data used for this paper are available in the supplementary materials (Table S5 and Table S6)

Funding

Veterinary pathogen work was part funded by the Department for Environment Food and Rural Affairs (Defra) and the Scottish and Welsh Governments through project SE0566.

CRediT authorship contribution statement

Sara L. Gandy: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Faye V. Brown:** Writing – review & editing, Investigation. **Nicola J. Jones:** Writing – review & editing, Investigation. **Sarah M. Biddlecombe:** Writing – review & editing, Investigation. **Georgia Kirby:** Writing – review & editing, Investigation. **Colin J. Johnston:** Writing – review & editing, Investigation. **Kayleigh M. Hansford:** Writing – review & editing, Conceptualization. **Alexander G.C. Vaux:** Writing – review & editing, Investigation. **Ternenge T. Apaa:** Writing – review & editing, Investigation. **Nicholas Johnson:** Writing – review & editing, Investigation. **Jolyon M. Medlock:** Writing – review & editing, Supervision, Data curation, Conceptualization, Investigation.

Acknowledgement

We would like to thank Forestry England for giving us permission to conduct our work and Matthias Alfredsson (Icelandic Institute of Natural History) for field assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2025.102541.

Data availability

All data related to the manuscript are in the supplementary materials

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