The roles of contrasting host types on the environmental abundance of *Anaplasma phagocytophilum*, an emerging zoonotic pathogen

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Abstract

Fundamental knowledge of the role of hosts in shaping vector-borne pathogen abundance is critical to understanding the ecology of these disease systems; yet the roles can be challenging to tease apart, especially for pathogens vectored by generalist feeders with multiple hosts. In this study, we aimed to quantify the relative contributions of hypothesised pathogen transmission hosts (deer and sheep) and non-transmission hosts (birds and rodents) to the environmental abundance of

Anaplasma phagocytophilum (ecotypes I and II) in *Ixodes ricinus* ticks*. Anaplasma phagocytophilum* is a zoonotic pathogen, vectored by *I. ricinus*, that causes tick-borne fever in livestock and anaplasmosis in humans. We collected data on *A. phagocytophilum* prevalence and environmental hazard (density of infected ticks at 40 sites), sheep presence (at 40 sites) and relative abundance indices of deer (at 40 sites) rodents (at 20 sites) and birds (at 10 sites) in northwest Scotland between 2018 and 2020. As predicted, deer were positively associated with *A. phagocytophilum* prevalence and hazard, indicating their potential role as pathogen transmission hosts. In contrast, bird abundance was negatively associated with *A. phagocytophilum* prevalence, indicating that higher bird densities may divert more ticks away from feeding on transmission hosts, thus reducing *A. phagocytophilum* prevalence. This highlights the importance of including non-transmission hosts when examining environmental disease risk factors. Sheep presence and rodent abundance index did not significantly influence *A. phagocytophilum* prevalence and hazard at our sites. Our findings could have implications for conservation and disease mitigation as land management practices that reduce deer density and promote bird abundance could reduce *A. phagocytophilum* prevalence and hazard.

Keywords: deer, birds, rodents, sheep, *Ixodes ricinus*, ticks

Introduction

Vector-borne diseases, which cause 700,000 human deaths worldwide each year (World Health Organisation, 2024), rely on vectors (often arthropods) to transmit the disease-causing pathogens between animal or plant hosts. (Medlock and Leach, 2015; Rulli *et al.*, 2025). The ecologies of vector-borne pathogens are often highly complex, especially those involving generalist vectors which feed on multiple host species. The relative abundance of different host species will partly determine both the proportion of vectors that are infected with the pathogen (pathogen prevalence) and the density

of infected vectors in the environment (environmental hazard of a disease) (Ostfeld and Keesing, 2000). Quantifying the role of multiple hosts can be challenging yet crucial for understanding the ecological mechanisms driving vector-borne pathogen prevalence and hazard. It is particularly important to understand the role of a range of different hosts, as some host species can contribute to higher pathogen prevalence in vector populations while other hosts may contribute to reducing pathogen prevalence (Ostfeld and Keesing, 2000). For example, some species of birds and small mammals can transmit *Borrelia burgdorferi* sensu lato (the complex of bacteria which causes Lyme disease in humans), and higher densities of these hosts are associated with higher prevalences of *B. burgdorferi* s.l. within tick populations (de Boer et al., 1993; Tälleklint and Jaenson, 1994; Humair, 2002; Dumas et al., 2022). In contrast, deer are not able to transmit *B. burgdorferi* s.l. (Tälleklint and Jaenson, 1994; Kurtenbach et al., 2002) and higher densities of deer are often associated with a lower proportion of ticks infected with *B. burgdorferi* s.l., because more juvenile ticks feed on the deer instead of on transmission hosts (Rosef, Paulauskas and Radzijevskaja, 2009; Vourc'h *et al.*, 2016; Gandy *et al.*, 2021; Gandy, Kilbride, *et al.*, 2022). To add further complexity, it can be possible for some types of host to reduce pathogen prevalence while increasing environmental disease hazard. For example, although deer can reduce the proportion of ticks infected with *B. burgdorferi* s.l., their role in feeding large numbers of ticks can result in a neutral or even positive effect on the density of infected ticks (Gandy et al. 2021, 2022). This phenomenon of non-transmission hosts playing an important role in shaping pathogen prevalence and disease hazard is not specific to the *B. burgdorferi* s.l. system and has been shown in other tick-borne disease systems (Gilbert *et al.*, 2001; Rulli *et al.*, 2025).

Anaplasma phagocytophilum is a tick-borne pathogen complex for which there is a gap in knowledge of how both transmission and non-transmission hosts shape prevalence and hazard (Sanchez et al., 2016). This is despite *A. phagocytophilum* having substantially higher prevalences in host seeking ("questing") ticks than *B. burgdorferi* s.l. in some areas (Rosef et al. 2009; Olsthoorn et al., 2021; Gandy, Hansford, et al., 2022). *Anaplasma phagocytophilum* is an emerging zoonotic pathogenic

complex of gram-negative, intracellular bacteria of both public and veterinary health importance. It causes disease in a wide range of vertebrates (Karshima *et al.*, 2023) including tick-borne fever in domestic ruminants (Stuen *et al.*, 2006; Bianchessi *et al.*, 2023) and granulocytic anaplasmosis in horses (*Equus ferus caballus) (Gussmann et al., 2014)* and dogs (*Canis lupus familiaris*) (El Hamiani Khatat *et al.*, 2021). The pathogen complex is genetically diverse with multiple variants that have been grouped into four (possibly five) distinct “ecotypes" (Bown et al., 2009; Stuen, Granquist and Silaghi, 2013; Jahfari et al., 2014; Santos et al., 2018).

In Europe, ecotypes I and II are vectored primarily by *Ixodes ricinus* ticks (*Ixodida: Ixodidae, Linnaeus*; Karshima *et al.*, 2023), the most widespread and abundant tick species in Europe (Barandika *et al.*, 2006; Medlock *et al.*, 2013; Gilbert, 2021; Gray, Kahl and Zintl, 2021). *Ixodes ricinus* is a generalist feeder which takes blood meals from most terrestrial vertebrate species (Gern and Humair, 2002; Hofmeester et al., 2016a; Gray, Kahl and Zintl, 2021; Fabri et al. 2022), enabling it to transmit pathogens between different host species. *Ixodes ricinus* has three active life stages: larva, nymph and adult and each stage requires a blood meal, before moulting to the next life stage (in the case of larvae or nymphs) or laying eggs (in the case of adult females) (Herrmann and Gern, 2015; Gray, Kahl and Zintl, 2021). Larvae and nymphs commonly feed on small, medium and large sized hosts (Hofmeester *et al.*, 2016b; Takumi, Sprong and Hofmeester, 2019a), whilst adult females rely on larger hosts such as deer or sheep (*Ovis aries*; (Herrmann and Gern, 2015; Gray, Kahl and Zintl, 2021). Ecotypes III and IV, associated with small mammals and birds respectively (Jahfari et al., 2014; Fabri et al., 2022), are rarely detected in *I. ricinus* ticks (Jahfari et al., 2014; Stigum et al., 2019; Takumi et al., 2021; Fabri et al., 2022) and ecotype V has been so far identified only in *I. ventalloi* ticks from southern Europe (Santos et 136 al., 2018). It therefore appears to be *A. phagocytophilum* ecotypes I and II exclusively that are vectored by *I. ricinus* (Jahfari et al., 2014) and, thus, our study is concerned only with ecotypes I and II.

Anaplasma phagocytophilum has often been reported in the tissues of a range of cervid species and wild and domestic ruminants (Stuen, Granquist and Silaghi, 2013; Jahfari *et al.*, 2014; Stigum *et al.*, 2019a; Remesar *et al.*, 2022). In addition, higher *A. phagocytophilum* prevalences in *I. ricinus* have been linked with higher densities of, or presence of, cervids, particularly red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) (Rosef et al., 2009; Stigum et al., 2019; Fabri et al., 2021, 2022; Takumi et al., 2021), or domestic ruminants (usually sheep; Ogden et al., 2003; Perrin, 2017; Gandy, Hansford, et al., 2022; Bianchessi et al., 2023). This suggests that cervids and ruminants can be important reservoir hosts. Specifically, ecotype I is the ecotype primarily (but not exclusively) detected in red deer, fallow deer and ruminants, whereas ecotype II is the ecotype primarily (but not exclusively) detected in roe deer (Jahfari *et al.*, 2014; Stigum *et al.*, 2019). Therefore, red and fallow deer and ruminants are likely to be amongst the key transmission hosts for ecotype I, while roe deer are considered to be the main transmission hosts for ecotype II (Hamšíková et al., 2019; Stigum et al., 2019; Fabri et al., 2024).

As well as being possible transmission hosts for ecotypes I and II, larger hosts, such as red and roe deer, are often the main drivers of *I. ricinus* tick density (Gray, 1998; Gilbert et al., 2012; Pacilly et al., 2014; Mysterud et al., 2016; Fabri et al. 2021). This is because they provide blood meals for large numbers (often over 100 per individual) of all *I. ricinus* tick stages (Kiffner *et al.*, 2010). In particular, deer are among the most important hosts that feed adult *I. ricinus*, which can then proceed to reproduce, hence deer act as ”tick reproduction hosts” (Gray, 1998; Ruiz-Fons *et al.*, 2006; Kiffner *et al.*, 2010; Opalińska *et al.*, 2021). Hence, as well as augmenting *A. phagocytophilum* prevalence, we might expect deer to also augment the environmental hazard of *A. phagocytophilum* (Takumi, Sprong and Hofmeester, 2019; Takumi, Hofmeester and Sprong, 2021; Fabri *et al.*, 2022, 2024). Similarly, ruminants, such as sheep and cattle can also play a role in maintaining both tick densities and prevalence of *A. phagocytophilum* ecotype I if they have not been treated with acaricide (Laurenson *et al.*, 2007), and so could also play a role in shaping *A. phagocytophilum* hazard.

Anaplasma phagocytophilum ecotypes I and II are rarely detected in rodents and never so far in birds (Jahfari *et al.*, 2014) and it is assumed that rodents and birds are not transmission hosts. No previous empirical studies, to our knowledge, have analysed data of non-transmission hosts to assess risk factors for the prevalence or environmental hazard of *A. phagocytophilum.* However, since rodents and birds can be important hosts for larval and nymphal *I. ricinus* (Gray, Kahl and Zintl, 2021), but are assumed to not infect feeding *I. ricinus* with ecotypes I or II, we might expect higher rodent or bird abundances to be associated with lower prevalence of *A. phagocytophilum* in questing *I. ricinus* (Fabri *et al*. 2022). This is because, where more larval and nymphal ticks feed on non-transmission hosts instead of on transmission hosts, a higher proportion of the tick population remains uninfected when they emerge as questing nymphs and adults several months later.

In this study, we aimed to test the roles of transmission hosts and, importantly, non-transmission hosts in shaping the prevalence and environmental hazard of *A. phagocytophilum* in questing *I. ricinus* ticks. As *I. ricinus* ticks vector ecotypes I and II, these are the ecotypes inferred when we refer to *A. phagocytophilum* and are the focus of this study. Specifically, we predicted that areas with higher densities of transmission hosts (deer and ruminants) should have higher prevalences and hazards of *A. phagocytophilum* in questing *I. ricinus* ticks (Ogden *et al.*, 2003; Rosef et al., 2009; Perrin, 2017; Stigum et al., 2019; Gandy, Hansford, et al., 2022; Bianchessi et al., 2023). We further predict that areas with higher densities of non-transmission hosts (rodents and birds) should have lower prevalences of *A. phagocytophilum* in questing *I. ricinus* ticks. Their effect on hazard, however, is difficult to predict because it also depends on their contribution to the *I. ricinus* population.

We tested these predictions by estimating the prevalence and environmental hazard of *A. phagocytophilum* in questing *I. ricinus* ticks and the relative abundance indices of deer, sheep, birds, and rodents, in Wester Ross, northwest Scotland, UK. We surveyed in a range of habitats to ensure a wide variation in host densities between sites.

Materials and methods

Survey design

The study region was in Wester Ross in the northwest of Scotland (Figure 1). This is a remote, scenic area, popular with tourists, with hills that rise from sea level on the coastline up to 1000 m elevation. The region comprises a mosaic of forests, rough upland grassland/moorland and montane habitats, with extensively grazed livestock (primarily sheep with small numbers of cattle) and scattered small human settlements. Cervids comprise mainly red deer; roe deer are also present at lower densities. The climate is temperate maritime, characterised by high rainfall (2300 mm per year), cool summers (mean maximum 18.5^{o}C in July) and mild winters (mean minimum 1.3^{o}C in January) (Met Office 2026).

We surveyed 40 sites from 2018-2020 inclusive for the prevalence and environmental hazard of *A. phagocytophilum* in questing *I. ricinus* ticks and the relative abundance indices of deer (red and roe) and presence of ruminants (sheep; no cattle or goats were present at these sites). Relative abundance indices of rodents and birds were estimated at a subset of 20 and 10 of these sites respectively, due to the resource intensity of the methods involved (Appendix Table 1). The surveyed sites comprised even numbers from each of five habitats representative of the main land cover types in the area: mature birch woodland, mature spruce woodland, mature pine woodland, young pine woodland and open moorland (Appendix Table 1). These were chosen specifically to ensure a wide range of population densities for each of the host types, to better test our predictions about the effect of each host type. The sites encompassed an area of approximately 75 km x 75 km, all at low elevation and sharing a similar climate, with adjacent sites being 0.5 km to 9 km apart (Figure 1).

Anaplasma phagocytophilum environmental hazard

Ixodes ricinus were collected and their density was estimated using blanket drag transects (Falco and Fish, 1992), which involved dragging a 1 m × 1 m blanket material over the ground vegetation for 10 m (Gilbert, 2010). Twenty transects were conducted for each site visit in a manner that was representative of the range of micro-habitats present in each site, under the condition that transects were separated by at least 20 m. All 40 sites were surveyed in 2018 over three visits: in spring (May), mid summer (June/July) and late summer (August), to cover the main Scottish tick activity season (MacLeod, 1939; Lees and Milne, 1951). In 2019 tick collection and surveys were conducted at a subset of 10 of the 40 sites (for which bird abundance index was estimated) over two visits (spring and late summer). In 2020 tick collection and surveys were conducted at a subset of 20 of the 40 sites (for which rodent abundance index had been estimated the year before) over one visit (late summer). This was due to Covid-19 movement restrictions. All nymphal and adult ticks were counted from each transect and collected into 1.5 mL Eppendorf tubes filled with 70% ethanol for later pathogen analysis (see "*Anaplasma phagocytophilum* prevalence" section below for methods).

Environmental hazard is the density of infected tick stages that pose a pathogen hazard to hosts and was thus calculated as the number of infected nymphs and adults per 10 m^2, i.e. per transect. Both nymphs and adults were used because both stages can be infected with *A. phagocytophilum*, posing an "environmental hazard" and both stages bite cervids and ruminants, the transmission hosts that can become infected. Additionally, infection was acquired (or not) when these nymphs and adults fed as larvae and nymphs (respectively) the previous year (or months); larvae and nymphs feed on all host types (cervids, ruminants, rodents and birds), and our aim was to link these four host categories with pathogen infection in all relevant tick stages that contribute to the transmission cycle and pose a hazard.

As the blanket dragging method can be influenced by vegetation density (Ruiz-Fons and Gilbert, 2010), we recorded ground vegetation height-density at the beginning, middle, and end of each

transect by using a sward stick with coloured tape placed every 5 cm in height. Ground vegetation height-density was estimated by counting how many bands could not be seen when the stick was placed in the vegetation at arm's length. The average value for each transect was calculated to produce a transect-level index of ground vegetation height-density (Gilbert, 2010). Air temperature at 15 cm above the ground was recorded using a temperature-humidity pen (Extech 445580) at the beginning and end of each site visit. The average of those two measurements at the site-visit level was used so that we could account for temperature-dependent variation in tick activity (Gilbert, Aungier and Tomkins, 2014). Since both temperature and ground vegetation height-density index increase throughout our survey season (from May through August), these variables also help account for variation in tick counts over the season.

Anaplasma phagocytophilum prevalence

Nymph and adult *I. ricinus* ticks were washed in distilled water, dried, and individually stored at -20°C until DNA extraction was conducted by boiling individual ticks in 0.7 M ammonium hydroxide as described by Wielinga et al., (2006). Extracted DNA samples were tested for the presence of *A. phagocytophilum* using targets to msp2 (Courtney *et al.*, 2004) genes using a real-time PCR (qPCR) protocol (Courtney *et al.*, 2004). The qPCRs were carried out on a LightCycler 480 (Roche Diagnostics Nederland B.V., Almere, the Netherlands) in a 20 µL volume, containing iQ Multiplex Powermix, 3 µL of sample, and 0.2 µM of primers and different concentrations of probes. Every PCR plate included positive controls and negative water controls. Samples positive for *A. phagocytophilum* were subjected to a qPCR protocol distinguishing between ecotypes I and II by use of two different probes as described (Gandy, Hansford, *et al.*, 2022). For confirmation of the ecotype specific qPCRs, *A. phagocytophilum*-positive samples were also sequenced to determine the ecotype, using conventional PCR followed by Sanger sequencing with the GroEL region according to previously published protocols (Jaarsma *et al.*, 2019). *Anaplasma phagocytophilum* prevalence was estimated as the proportion of *I. ricinus* (nymphs plus adults) tested that were infected with either of the *A. phagocytophilum* ecotypes.

Both prevalence and hazard were estimated using both ecotypes together because (i) our deer abundance index was a combination of both red and roe deer; and (ii) both ecotypes are not transmitted by rodents and birds, so we expect areas with more rodents and birds to have lower prevalence of both ecotypes. Hereafter, for the purposes of our study, we use the term *A. phagocytophilum* to mean both ecotypes I and II, unless otherwise specified.

Deer abundance index and sheep presence

At all 40 sites we counted the number of deer dung piles over the same 10 m x 1 m transects over which ticks were surveyed (Gilbert 2010). Deer dung counts were conducted at all sites during the first visit of the year for each site, i.e. in May, when ground vegetation is shorter and deer dung detection less prone to error. However, in 2020 all surveys were delayed until August due to Covid-19 movement restrictions (Appendix Table 1). An index of relative deer abundance was estimated at the site-year level by averaging the number of deer dung piles per transect for each site for each year. Both red and roe deer were present in the study region. However, due to the difficulty in accurately distinguishing between young red deer and roe deer dung, we combined counts from both species into one deer abundance index.

Sheep presence or absence at each site was established through communication with landowners before visiting each site and confirmed visually during each visit. No other ruminant livestock (cattle or goats) were present at any of our sites. Sheep presence/absence was a site-level parameter.

Bird abundance index

Passerine birds were live-trapped using mist nets at a subset of 10 sites (five mature Scots pine woodlands and five mature birch woodlands) in 2018. It was not possible to survey birds at more sites as the method is highly resource-intensive and requires trained and licenced personnel volunteering their time. At each site, two 18 m-long mist nets were deployed, approximately 200 m

apart. The songs of 26 different local bird species were played at both net locations. Mist nets were deployed at each site for four hours in the morning and four hours in the evening of the same day and each net was checked for birds a minimum of once every 30 minutes. A numbered metal ring (band) was fitted to the leg of each bird which ensured we could identify and discount any individuals that were captured more than once. Mist netting was led by an experienced, qualified, licenced bird handler and mist-netter under British Trust for Ornithology licence number A4488 (ethical approval is not required for this by the University of Glasgow). For an index of relative abundance of birds to be relevant to the *I. ricinus-A. phagocytophilum* system it is important to include only those bird species that carry *I. ricinus*. Therefore, we used the total number of Eurasian chaffinches (*Fringilla coelebs*), European robins (*Erithacus rubecula*), dunnocks (*Prunella modularis*), great tits (*Parus major*), and northern wrens (*Troglodytes troglodytes*) captured at each site. These were the five species caught that were found to carry ticks in this study and were by far the most frequently caught. As they are ground-foragers, these species are known to be amongst those passerine bird species most implicated as hosts of *I. ricinus* in Scotland (James *et al.*, 2011) and elsewhere in Europe (Keve, Sándor and Hornok, 2022). While a small number of individuals from other species were caught, we did not include them in analyses as these did not carry ticks at our sites, are not routine ground-foragers and are not reported to be important hosts to *I. ricinus* in Scotland (James et al. 2011) or elsewhere in Europe (Keve et al. 2022).

Rodent abundance index

Rodents were live-trapped using Longworth traps at a subset of 20 of the 40 sites, limited by the number of personnel and traps available, as it was important to conduct trapping at all sites in a short period of time due to seasonal differences in rodent abundance (Butet, Paillat and Delettre, 2006). All 20 sites were surveyed for rodents in August/September 2019; and eight of these sites had also been surveyed for rodents in August/September 2018 (Appendix Table 1). Fifty traps per site

were laid out on 40 m x 40 m grids, where two traps were deployed at every 10 m point on the grid. Traps were deployed over two consecutive nights per site. Each trap was bedded with hay and baited with oats and seeds. The traps had holes in them which would allow shrews (*Sorex spp.*) to escape as per UK small mammal live trapping regulations. Traps were deployed in the evening at 8 pm, checked and closed the following morning at 8 am, and re-deployed at 8 pm. All captured mice and voles had their fur clipped to enable identification of re-trapped individuals. Individual rodents caught more than once, and traps that had failed or been set off by other means, were excluded from estimating the rodent abundance index. The abundance index was the number of newly captured individuals per 100 trap-nights for each site.

Rodents of three species were captured: bank voles (*Myodes glareolus*), field voles (*Microtus agrestis*), and wood mice (*Apodemus sylvaticus*). Since all three rodent species are considered non-transmission hosts for *I. ricinus*-associated *A. phagocytophilum* (i.e. both ecotypes I and II), we predict they would have similar roles in shaping *A. phagocytophilum* prevalence and hazard. Therefore, the number of captures for each species was combined into a single rodent abundance index for testing our predictions about the role of rodents, as one of the non-transmission host types, on *A. phagocytophilum*.

There was little evidence of rodent abundance varying consistently between the two rodent survey years (80 per 100 trap-nights in 2018 and 92 per 100 trap-nights in 2019); therefore, at sites where rodents were surveyed in both years, an average was taken between the two years to create a more robust estimate. Rodent abundance index was a site level variable, with one value per site (as was also the case for birds and sheep).

Statistical analysis

All statistical analyses were conducted using R (R core Team, version 2022.12.0) and we used Generalised Linear Mixed Effect Models (GLMMs) to test our predictions. For all models, we first

tested for potential collinearity between our explanatory variables by calculating the variance inflation factors (VIFs) (Zuur et al., 2009). Collinearity (VIF score >4) with bird and rodent abundance indices, was detected for habitat type. Checks for over-dispersion and zero-inflation were carried out on the distributions of the response variables using the zero-inflation test and the over-dispersion test in the DHARMa package (Hartig, 2016). These compared fitted against simulated residuals and enabled us to specify the appropriate distribution in the models: all hazard data were over-dispersed beyond the Poisson, so negative binomial distribution was specified for all hazard models. A zero-inflation factor was not required in the models.

Because data on rodents and birds were collected at a subset of sites, we used separate models to test for the effects of (i) deer and sheep (using data from all 40 sites) (ii) birds, (using data from 10 sites) and (iii) rodents (using data from 20 sites) on *A. phagocytophilum* prevalence and hazard.

Association of host types with *A. phagocytophilum* prevalence in *I. ricinus*

In each of the three models (deer and sheep; birds; rodents), the response variable was the prevalence of *A. phagocytophilum* expressed as the number of nymphs and adults testing positive for *A. phagocytophilum* (ecotypes I or II) over the total number of ticks that were negative, modelled at the site visit level using the lme4 package (Bates *et al.*, 2015). We used binomial GLMMs with logit links. The binomial response used a two-column matrix of counts (number of positive ticks and number of negative ticks), implemented via cbind(positive, negative). This formulation is an extension of the binomial framework and is mathematically equivalent to modelling individual-level binary outcomes, but aggregated at the site visit level. The model therefore estimates the probability of infection (prevalence) while appropriately accounting for varying sample sizes of ticks tested across sites. This approach of using a binomial model with a cbind response is useful when modelling proportions or prevalence data, as it avoids the loss of information that would occur if site-level proportions were analysed without incorporating the underlying counts/sample size.

The first model was designed to test the prediction that deer and sheep would augment *A. phagocytophilum* prevalence and therefore the key fixed effects of interest were deer abundance index (average number of deer dung piles per transect, at site year level) and sheep presence/absence at the site level (but not rodents or birds, in order to use data from all 40 sites). Habitat (open moorland, young Scots pine, mature Scots pine, mature birch, and mature Sitka spruce) was also included as a fixed effect.

The second and third models were designed to test predictions that birds and rodents respectively can reduce *A. phagocytophilum* prevalence. Therefore, the key fixed effects of interest were bird abundance index from the 10 bird survey sites for the bird model; and rodent abundance index from the 20 rodent survey sites for the rodent model. Deer index was also included as a fixed effect in both bird and rodent models, as we expected deer to also influence prevalence. The models would not run when sheep presence was included, presumably due to small sample sizes of bird and rodent sites, coupled with very few bird (n = 3) and rodent (n = 5) sites with sheep present. Habitat was not included as a fixed effect because it covaried with both bird and rodent abundance indices (VIF > 4).

All prevalence models included season of tick survey (categorised as spring, mid summer or late summer) as an additional fixed effect to account for any seasonal effects on prevalence, such as might operate through seasonal patterns of tick activity, host abundance.

All three models included year (2018, 2019, 2020) nested within site as a random effect, since prevalence data from all 3 years of sampling were included in the models and each site was visited across multiple years.

Association of host types with environmental hazard of *A. phagocytophilum* in *I. ricinus*

To test the effects of (i) deer and sheep, (ii) birds and (iii) rodents on the density of ticks infected with *A. phagocytophilum* (environmental hazard) we used three negative-binomial GLMMs with log links. A negative-binomial distribution was specified in the models because tests using the DHARMa package indicated overdispersion beyond the Poisson (Hartig, 2016). In each GLMM, the response

variable (environmental hazard) was expressed as the number of infected ticks (nymphs and adults) with an offset for the area surveyed, modelled at the site visit level.

As per our prevalence models, host indices were included as fixed effects in each GLMM as follows: the first model included both deer abundance index and sheep presence at all 40 sites, as well as habitat. The second and third models included bird and rodent abundance indices respectively, as well as deer abundance index, as deer are expected to influence both components of hazard: prevalence and density of ticks. As for the prevalence models, the hazard models using the smaller data sets for rodents and birds would not run with sheep presence/absence, so sheep could not be included in the bird and rodent models. Habitat was not included as a fixed effect because it covaried with both bird and rodent abundance indices (VIF > 4).

All hazard models included ground vegetation height-density index, air temperature and season as fixed effects. The inclusion of ground vegetation height-density index accounted for its potential effect on the efficiency of the blanket drag method (Ruiz-Fons and Gilbert, 2010). Air temperature affects tick questing activity (Gilbert, Aungier and Tomkins 2014), and therefore, needed to be accounted for. Including season (spring, mid summer, late summer) accounts for seasonal variation in tick activity. These three variables affect the number of ticks counted per 10 m^2 which is a component of environmental hazard.

As with the prevalence models, the random effect was year nested within site.

Model selection

Model selection was applied to each of the saturated models for both prevalence and hazard using the dredge function from the MuMIn package (Kamil Bartoń, 2010) which produces a list of candidate models, with differing fixed effect configurations, ranked according to corrected Akaike information criterion (AICc) where a smaller AICc value indicates a better fit (Brewer, Butler and

368 Cooksley, 2016). AICc is a modified version of AIC developed for use with smaller sample sizes or
369 when the number of fitted variables is a moderate-large proportion of the sample size (Hurvich and
370 Tsai, 1989), as is the case in our study.

371 Models with an AICc score within two points (ΔAICc < 2) of each other are generally considered to be
372 of similarly good fit (Mazerolle, 2006; Fabozzi *et al.*, 2014). Therefore, we did not necessarily select
373 the model with the lowest AICc score; if there was an occasion when a candidate model had an AICc
374 within 2 points of the model with the lowest AICc, we selected that model (Mazerolle, 2006; Fabozzi
375 *et al.*, 2014). In other words, a fixed effect was omitted from a selected model if its inclusion
376 increased the AICc score by more than two. Diagnostics from all final selected models were checked
377 using the DHARMa package (comparing fitted and simulated residuals using QQ plots).

378 Results

379 Over the three years of data collection (2018 – 2020), 9365 *I. ricinus* were collected from the 40 sites:
380 8867 nymphs and 498 *I. ricinus* adults. The overall average infection prevalence of *A.*
381 *phagocytophilum* was 5.3% [95% CI: 4.8 - 5.8] (496/9365) *I. ricinus* (nymphs and adults). The
382 environmental hazard of *A. phagocytophilum* per site visit was, on average, 11.2 infected *I. ricinus*
383 (nymphs and adults) per km^2 [95% CI: 7.7 - 14.7; range 0.0 - 111.3]. Out of the 496 ticks that tested
384 positive for *A. phagocytophilum,* 346 could be identified to ecotype: 86% (297/346) were ecotype I,
385 and 14% (49/346) were ecotype II.

386 The mean deer abundance index was 0.9 deer dung piles per 10 m^2 (SD = 1.2; range 0.0 - 5.8) and
387 sheep were present at 20% (8/40) of sites. The total number of individual birds (from the five species
388 retained: Eurasian chaffinches, Eurasian wrens, European robins, dunnocks, and great tits) counted in
389 mist-nets was 77 over the 10 sites where birds were surveyed, and the mean bird abundance index
390 per site was 4.2 (SD = 3.5; range 0 - 10). The total number of individual rodents captured (combining

wood mice, bank voles and field voles) was 240 across the 20 sites where rodents were surveyed, and the mean rodent index per site was 5.9 per 100 trap-nights (SD = 4.2; range 0.0 - 13.6) (Table 1).

Association of deer and sheep with *A. phagocytophilum* prevalence

Deer abundance index was strongly positively correlated with *A. phagocytophilum* prevalence (p <0.001; Figure 2a; **Error! Reference source not found.**). The mean infection prevalence for *A. phagocytophilum* was, on average, 1.6 times higher in sites where sheep were present compared to where sheep were absent (Figure 3a); however, sheep presence was not retained in the selected model (Table 2). Habitat and season were also not retained in the selected model (Table 2).

Association of birds with *A. phagocytophilum* prevalence

Anaplasma phagocytophilum prevalence was negatively correlated with bird abundance index (p = 0.008; Figure 4a; **Error! Reference source not found.**). Deer abundance index and season were not retained.

Association of rodents with *A. phagocytophilum* prevalence

Rodent abundance index was not retained in the model (Figure 5a; Table 2). Deer abundance index was positively associated with prevalence (p = 0.004; Table 2). Season was not retained.

Association of deer and sheep with *A. phagocytophilum* hazard

Deer abundance index was strongly positively correlated with the environmental hazard of *A. phagocytophilum* (p = 0.002; Figure 2b; **Error! Reference source not found.**), while spring had the highest predicted *A. phagocytophilum* hazard (Table 3). Sites with sheep had 3.5 times the hazard of

sites without sheep (Figure 3b), but sheep presence was not retained in the selected model (Table 3). Habitat, temperature and ground vegetation were also not retained in the selected model.

Association of birds with *A. phagocytophilum* hazard

There was no statistically significant association between *A. phagocytophilum* hazard and bird abundance, although birds were retained in the model as including them did not increase AICc by more than 2 ($p = 0.22$; Figure 4b; Table 3). Temperature was negatively correlated with *A. phagocytophilum* hazard and spring was predicted to be the time of year with the highest hazard (Table 3). Ground vegetation and deer were not retained in the model (Table 3).

Association of rodents with *A. phagocytophilum* hazard

There was a positive association between the environmental hazard of *A. phagocytophilum* and rodent abundance index ($p = 0.02$; Figure 5b; Table 3) and with deer abundance index ($p = 0.005$; Table 3). Spring was predicted to have the highest hazard (Table 3). Ground vegetation and temperature were not retained (Table 3).

Discussion

In this study, we aimed to test the effect of two hypothesised types of transmission hosts, deer and sheep, and two hypothesised types of non-transmission hosts, birds and rodents, on the prevalence and hazard of *A. phagocytophilum* in questing *I. ricinus* ticks. As predicted, deer abundance index was positively associated with both the prevalence and hazard of *A. phagocytophilum*. Deer have previously been suspected to be transmission hosts for *A. phagocytophilum* (Rosef *et al.*, 2009; Stuen, Granquist and Silaghi, 2013; Hamšíková *et al.*, 2019; Stigum *et al.*, 2019), and are well known to be amongst the most important hosts driving *I. ricinus* populations (Gray, 1998; Gilbert *et al.*,

2012; Pacilly *et al.*, 2014; Mysterud *et al.*, 2016; Gray, Kahl and Zintl, 2021). Our results are consistent with a study from the Netherlands, which found that the density of *I. ricinus* nymphs infected with *A. phagocytophilum* was higher in areas where the relative availability of red deer, fallow deer (*Dama dama*), and roe deer (estimated based on camera and live trapping data) were also high (Takumi, Hofmeester and Sprong, 2021). Of direct importance to livestock health, deer presence has also been shown to influence *A. phagocytophilum* infection in cattle (Rousseau *et al.*, 2021). The results of our analyses are consistent with these previous studies and provide further evidence that deer can augment the prevalence and hazard of *A. phagocytophilum* ecotypes in *I. ricinus* ticks.

Our average *A. phagocytophilum* prevalence was 5.3% over all our 40 sites in northwest Scotland, which is higher than in northern England (4.7%) and much higher than in southern England (1.8%) (Gandy *et al*., 2022). This might suggest there could be a general trend of increasing prevalence from south to north in the UK, perhaps reflecting higher sheep and deer densities further north. At our 40 sites, 86% of successfully ecotyped *A. phagocytophilum* samples were ecotype I while 14% were ecotype II. These values are remarkable similar to a study across England and Wales, that found that positive samples comprised 86.8% ecotype I and 13.2% ecotype II (Gandy *et al*., 2022), suggesting these ecotype ratios may be typical across the UK. In our study region of Scotland, this likely reflects the greater abundance of sheep and red deer relative to roe deer, since red deer (and sheep) are associated mostly with ecotype I (Rosef *et al.*, 2009; Jahfari *et al.*, 2014; Stigum *et al.*, 2019; Fabri et al. 2021, 2022) while roe deer are associated mostly with ecotype II (Jahfari *et al.*, 2014; Hamšíková *et al.*, 2019; Stigum *et al.*, 2019). However, due to the difficulty in confidently distinguishing the dung of young red deer from dung of adult roe deer our deer abundance index was a combination of roe and red deer dung counts. It would be interesting to investigate the differential roles of red and roe deer separately where they co-occur by strategically selecting sites that encompass a range of red and roe deer density ratios and statistically analysing ecotype I separately from ecotype II. We might expect that areas with higher roe deer abundance, while having higher prevalence of ecotype II, might have lower ecotype I prevalence, if roe deer have poor transmission competency for ecotype I

(Fabri *et al*., 2024). This would require a reliable alternative method of assessing relative abundance, to avoid the issues associated with dung identification.

Since ruminants are considered transmission hosts for *A. phagocytophilum* (primarily ecotype I), we predicted that sheep present at our sites would have a positive influence on *A. phagocytophilum* in *I. ricinus* ticks. Previous research has demonstrated positive associations between sheep presence and *A. phagocytophilum* prevalence (Ogden et al., 2003; Perrin, 2017; Gandy, Hansford, et al., 2022; Bianchessi et al., 2023), and we found the mean infection prevalence and hazard for *A. phagocytophilum* was 1.6 and 3.5 times higher (respectively) in the sites where sheep were present compared to where sheep were absent. However, sheep presence was not retained in our selected models. This is likely to be because sheep were only present in eight of our 40 survey sites presence/absence data do not allow for analysis of a quantitative range in sheep densities. Furthermore, the role of sheep in shaping *I. ricinus* tick and tick-borne pathogen abundance can be strongly influenced by application of acaricides (Laurenson *et al.*, 2007). To better understand the role of sheep in influencing the ecology of *A. phagocytophilum*, analysis of *A. phagocytophilum* prevalence and hazard across a range of sheep densities would provide deeper insight. This would be particularly relevant for informing potential mitigation strategies for tick-borne fever in sheep, such as managing stocking densities, or targeting acaricide treatments depending on stocking density-related risk.

As predicted, we found a negative correlation between bird abundance and the prevalence of *A. phagocytophilum* in *I. ricinus,* an empirical association that has not been tested previously to the best of our knowledge. Since previous studies have not found *A. phagocytophilum* ecotypes I and II in bird tissue samples or ticks collected from birds (Jahfari *et al.*, 2014), it is likely that birds do not support transmission of *A. phagocytophilum* to *I. ricinus* (i.e. ecotypes I or II). Therefore, where there are higher densities of birds, a higher proportion of immature *I. ricinus* are likely to feed on birds instead of on pathogen transmission hosts, thus resulting in lower pathogen prevalence in the tick

population. This effect has been observed in relation to high densities of deer reducing *B. burgdorferi* s.l. prevalence in questing *I. ricinus* ticks, because deer can feed large number of ticks (Kiffner *et al.*, 2010; Mysterud, Hatlegjerde and Sørensen, 2014; Gray, Kahl and Zintl, 2021) but do not transmit *B. burgdorferi* s.l. (Rosef *et al.*, 2009; Vourc'h *et al.*, 2016; Gandy *et al.*, 2021; Gandy, Kilbride, *et al.*, 2022). Our finding that birds may reduce *A. phagocytophilum* prevalence further demonstrates the importance of including non-transmission hosts in analyses of pathogen ecology, even when individuals of those host species do not tend to carry large tick burdens (James *et al.*, 2011).

One potential alternative explanation for a negative relationship between birds and *A. phagocytophilum* prevalence could be if deer (that augment prevalence) are a confounding factor. Grazing pressure from high densities of deer can reduce ground vegetation and, thus, negatively affect the abundance of smaller vertebrates such as birds (Flowerdew, 2001; Buesching *et al.*, 2011; Crystal-Ornelas *et al.*, 2021; Gandy *et al.*, 2021). Therefore, a negative association between bird abundance index and prevalence could occur if sites with higher deer abundance have lower bird abundance. However, this is unlikely to be the case in our study, since we included deer in all the models, so that any such confounding effects were already statistically accounted for.

Despite birds being positively associated with *A. phagocytophilum* prevalence, we found no significant relationship with hazard. Birds can occur at high densities, and our results suggest that, at the population level, birds could be increasing *I. ricinus* densities. If birds increase tick densities but decrease *A. phagocytophilum* prevalence, this could result in no overall effect of birds on the environmental hazard of *A. phagocytophilum* (the density of infected ticks). A similar mechanism has been hypothesised for the effect of deer on *B. burgdorferi* s.l.: deer can reduce *B. burgdorferi* s.l. prevalence but, because they have positive effects on tick density, there can be no overall effect of deer on environmental hazard of Lyme disease (Gandy et al. 2022).

The likelihood of a bird becoming infested with ticks is largely dictated by the foraging, nesting, and roosting behaviours unique to each bird species (James *et al.*, 2011; Heylen, 2016). Therefore, the

514 contributions of birds to the prevalence and hazard of tick-borne pathogens is highly species
515 dependent, with ground-foraging birds such as song thrushes, (*Turdus Phylomelos*), and European
516 blackbirds, *(T. Merula*), being particularly implicated in carrying *I. ricinus* ticks and *B. burgdorferi* s.l.
517 (James et al. 2011; Heylen, 2016). European blackbirds and song thrushes were not caught at the 10
518 sites we used for surveying birds. Instead, the five species of bird included in our analysis were
519 Eurasian chaffinches, Eurasian wrens, European robins, dunnocks, and great tits. These species were
520 the most abundant at our sites and were those found to be carrying *I. ricinus* ticks. They are resident
521 year-round in the UK, abundant in a range of habitats, and are widely distributed across Europe
522 (Hewson *et al.*, 2007; Walker *et al.*, 2023). These species therefore have the potential to influence *A.*
523 *phagocytophilum* ecology in many regions and countries. Our results might suggest that conservation
524 measures that increase the abundance of ground-foraging birds, such as woodland regeneration,
525 may help to reduce the prevalence of *A. phagocytophilum*. However, without a matched reduction in
526 hazard, due to the role these bird species can play in feeding immature *I. ricinu*s ticks, an increase in
527 bird abundance may not result in reduced risk to livestock and humans. Increased bird abundance
528 can also be associated with an increase in the circulation of bird-associated genospecies of *B.*
529 *burgdorferi* s.l. (Heylen, 2016), one of which (*B. garinii*) can cause Lyme disease in humans (Baranton
530 *et al.*, 1992; Strle *et al.*, 2006). Therefore, it is possible that environmental conditions (such as
531 abundance of certain hosts) that decrease the prevalence of one pathogen can increase the
532 prevalence of another pathogen. Indeed, previous studies have reported a negative relationship
533 between the prevalences of *A. phagocytophilum* and *B. burgdorferi* s.l. (Knoll *et al.*, 2021; Gandy,
534 Hansford, *et al.*, 2022). Future research could test these relationships to develop a deeper
535 understanding of the role of birds and other hosts in *A. phagocytophilum* ecology, including the
536 trade-offs between prevalence and tick production to shape hazard, and trade-offs between different
537 tick-borne pathogens.

538 Against our prediction that rodents would, like birds, reduce *A. phagocytophilum* prevalence, we
539 found no evidence of an effect of rodents on prevalence. However, the abundance of rodents was

low in many of our sampled sites in our two years of sampling. It remains possible that rodents could be associated with lower *A. phagocytophilum* prevalence in *I. ricinus*, in regions and years with higher rodent abundance. Furthermore, the accuracy of live rodent trapping in reflecting true rodent abundance can vary with ground vegetation type, introducing error in rodent abundance estimates across different habitats (Gandy et al. 2022). Having said that, our rodent indices did seem reliable enough to reflect the role of rodents in feeding larval *I. ricinus* ticks, thus augmenting the density of nymphs (van Duijvendijk, Sprong and Takken, 2015; Cayol *et al.*, 2017), reflected by a significant positive association between rodent abundance and the environmental hazard of *A. phagocytophilum.*

Our study may have important implications for land management, conservation and disease mitigation strategies. Our findings suggest that measures to reduce the density of deer – a commonly used tool to aid habitat recovery and woodland regeneration - may cause a decrease in both the prevalence and hazard of *A. phagocytophilum*, thus reducing disease risk for humans and livestock. The same conservation or deer management tools can result in higher abundance of several bird species, which may further reduce *A. phagocytophilum* prevalence although, as found here, that will not necessarily result in reduced disease hazard due to the role of birds in feeding immature ticks. There is also the possibility that higher bird densities could lead to higher prevalence or hazard or other tick-borne pathogens which are transmitted by birds, such as *B. garinii* (Heylen, 2016).

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Availability of data:

The data that support the findings of this study are openly available in DRYAD at http://datadryad.org/share/LINK_NOT_FOR_PUBLICATION/EYNomWQl5K9M4lodGFyWzvxzgRDMf7egp_iJEBegOtg

Authors' contributions:

William McLellan collated, cleaned, analysed and interpreted the data and wrote the paper.

Sara Gandy supervised the data analysis and interpretation and contributed to writing the paper.

Jaboury Ghazoul gained the funding for, instigated and supervised the field data collection aspects.

Fanny Olsthoorn led all aspects of the field data collection.

Livia May helped lead on, and conducted, the bird data collection.

Jude Eze provided statistical advice and commented on paper drafts.

Hein Sprong instigated, funded, led and conducted the pathogen lab work and provided the Anaplasma and Borrelia data.

Mara Rocchi provided Anaplasma advice and commented on paper drafts.

Lucy Gilbert conceptualised the framework, supervised the data collation, analysis and interpretation, and contributed to writing the paper.

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Figure captions:

Figure 1: Map of study region within Scotland (inset) showing locations of the 40 study sites. The hosts (deer, sheep, birds and rodents) surveyed at each site are indicated by colour.

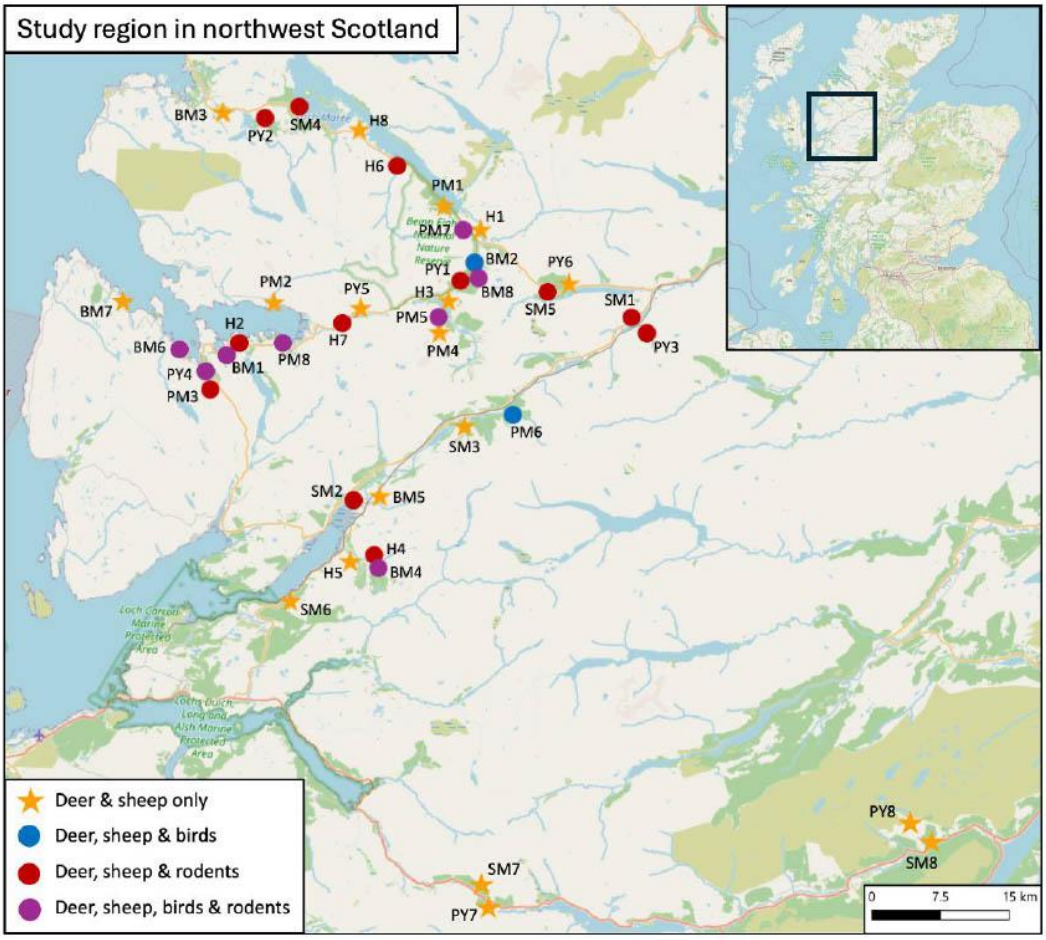
Figure 2. Output model predictions of the effect of deer (roe and red) abundance index (number of deer dung piles 10 per m^2) on (a) infection prevalence of *Ixodes ricinus* ticks (nymphs and adults) with *Anaplasma phagocytophilum* (%); and (b) environmental hazard of *A. phagocytophilum* (density of infected *I. ricinus* nymphs and adults per km^2) at 40 sites. The solid lines represent the predicted mean values, and the shaded areas display the 95% confidence intervals.

Figure 3. Relationship between sheep presence/absence at the site level and (a) infection prevalence of *Ixodes ricinus* ticks (nymphs and adults) with *Anaplasma phagocytophilum* (%); and (b) environmental hazard of *A. phagocytophilum* (density of infected *I. ricinus* nymphs and adults per km^2) at 40 sites. Unadjusted raw data are shown. The white boxes depict the interquartile range of prevalence values and the solid horizontal dark line within them represents the mean. The solid lines represent the predicted mean values, and the shaded areas display the 95% confidence intervals.

Figure 4. Output model predictions of the effect of bird abundance index (number of birds captured per site) on (a) infection prevalence of *Ixodes ricinus* ticks (nymphs and adults) with *Anaplasma phagocytophilum* (%); and (b) environmental hazard of *A. phagocytophilum* (density of infected *I. ricinus* nymphs and adults per km^2) at 10 sites. The solid lines represent the predicted mean values, and the shaded areas display the 95% confidence intervals.

Figure 5. Output model predictions of the effect of rodent abundance index (newly captured rodents trapped/ 100 trap-nights per site) on (a) infection prevalence of *Ixodes ricinus* ticks (nymphs and adults) with *Anaplasma phagocytophilum* (%); and (b) environmental hazard of *A. phagocytophilum* (density of infected *I. ricinus* nymphs and adults per km^2) at 20 sites. The solid lines represent the predicted mean values, and the shaded areas display the 95% confidence intervals. The solid lines represent the predicted mean values, and the shaded areas display the 95% confidence intervals. Note that rodents were not retained in the final selected model, so these predicted values were gained from the (not selected) model with rodents included.

883 **Figure 1.**



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886 **Figure 2.**

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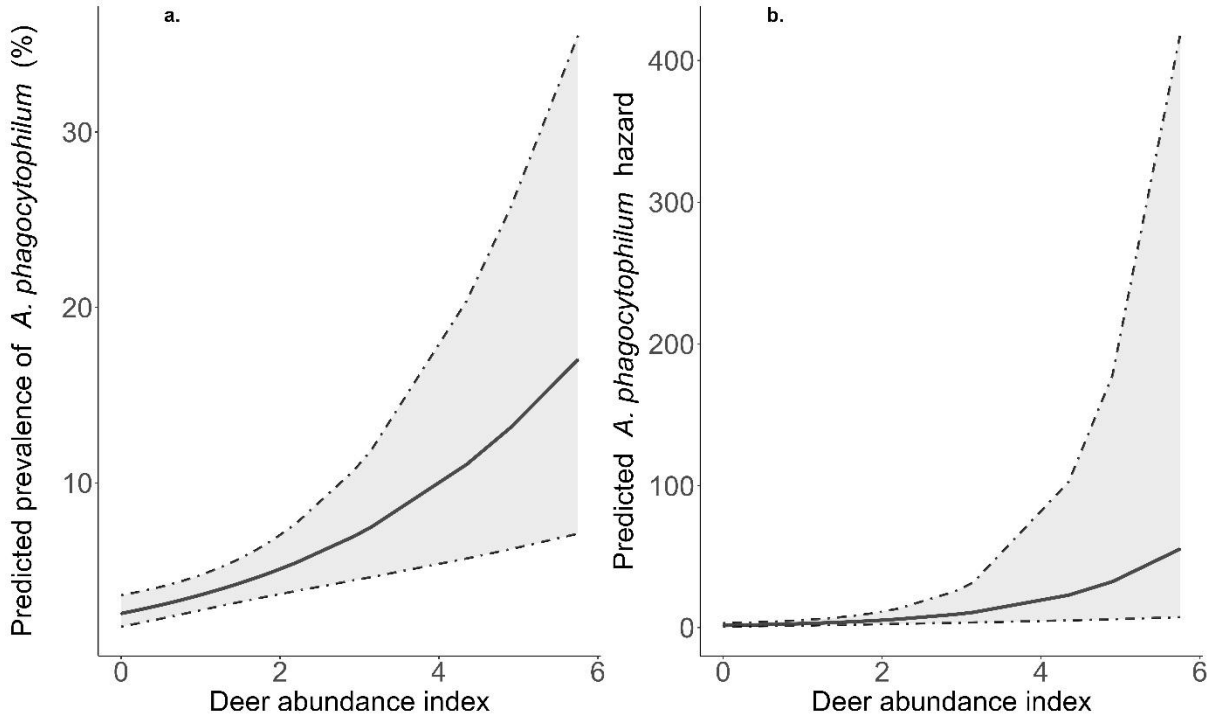

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890 **Figure 3.**

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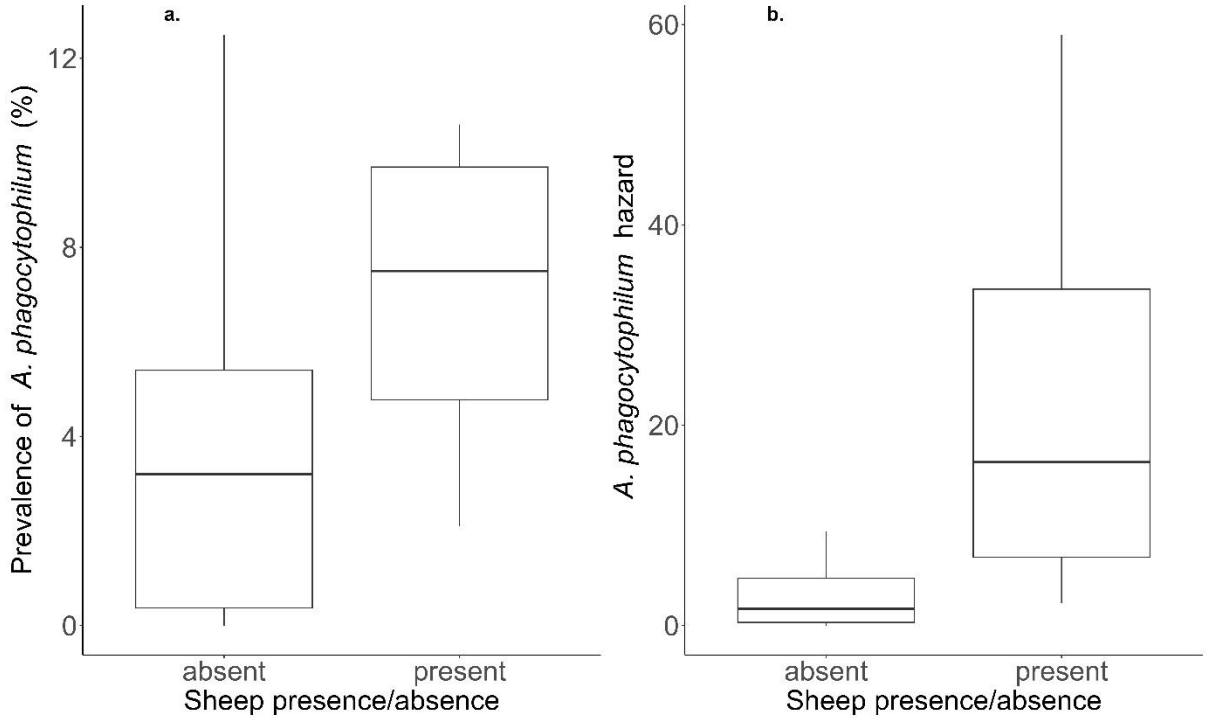

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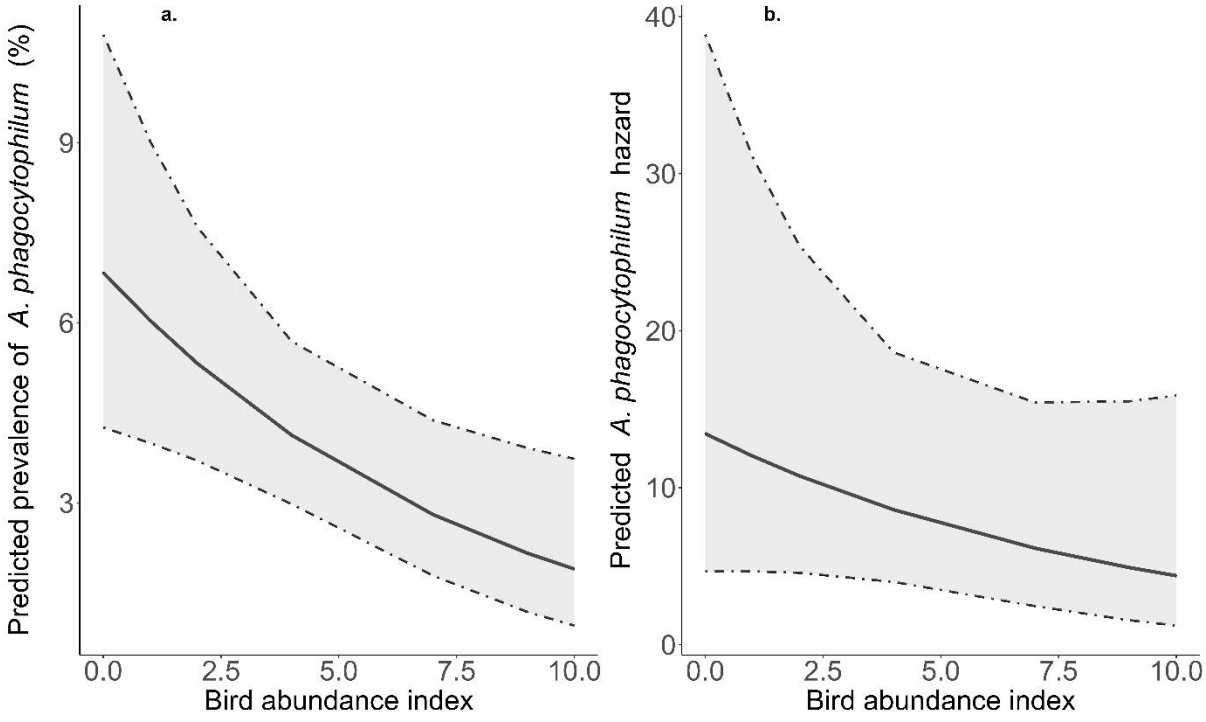
Figure 4.


a.
Predicted prevalence of A. phagocytophilum (%)
9
6
3
0.0
2.5
5.0
7.5
10.0
Bird abundance index
b.
Predicted A. phagocytophilum hazard
40
30
20
10
0
0.0
2.5
5.0
7.5
10.0
Bird abundance index

900 **Figure 5.**

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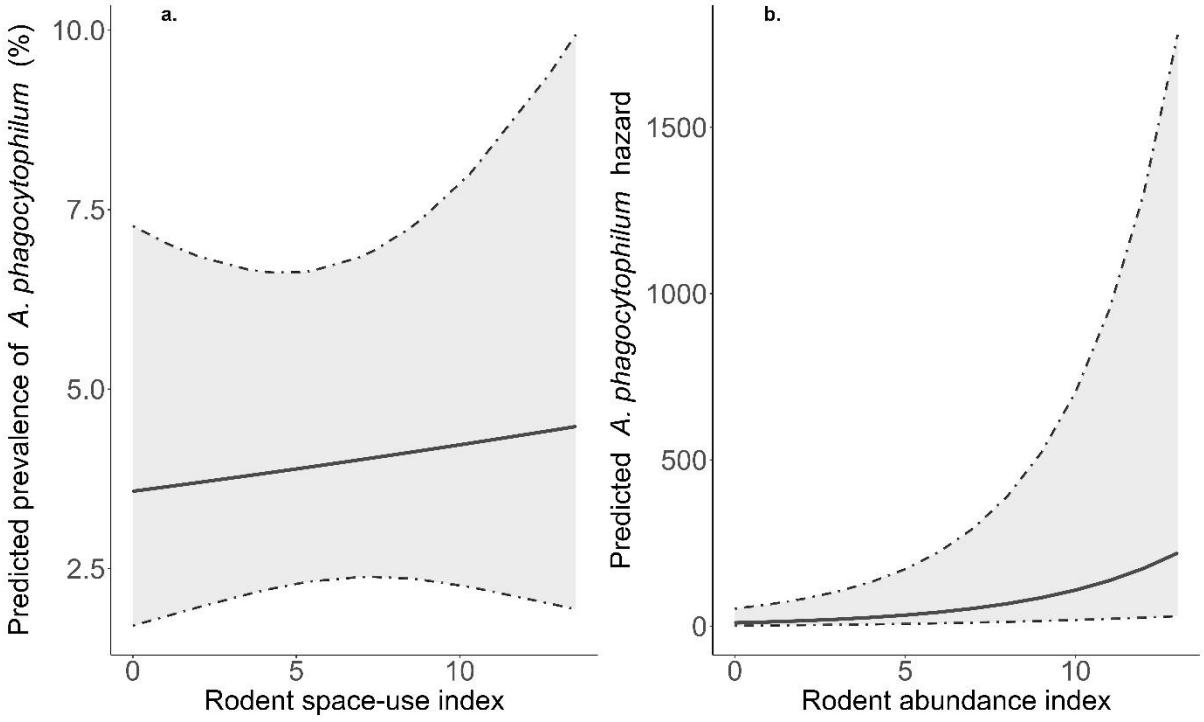

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Table legends:

Table 1: Summary data showing the mean values for each site of *A. phagocytophilum* prevalence and hazard, the bird and rodent indices and the mean deer abundance index for each site. BM indicates mature birch, PM is mature pine, PY is young pine, SM is mature spruce and H indicates upland moorland.

Table 2: Output from the generalised linear mixed effects models showing the effects of deer, birds and rodents on infection prevalence of *A. phagocytophilum* in questing *Ixodes ricinus* nymphal and adult ticks. Delta AICc is the change in AICc if the variable is removed (a negative value denotes a decrease in AICc score, indicating a better model fit; positive values indicate a worse fit). A variable was removed from the model if removing it caused an increase in AICc score of more than two. All variables initially entered into the global model are listed; those that were excluded during model selection are indicated by "Excluded from model".

Table 3: Output from the generalised linear mixed effects models showing the effects of deer, birds and rodents on the environmental hazard of *A. phagocytophilum* (density of infected *Ixodes ricinus* nymphal and adult ticks) ΔAICc is the change in AICc if the variable is removed (a negative value denotes a decrease in AICc score indicating a better model fit; positive values indicate a worse fit). A variable was removed from the model if removing it caused an increase in AICc score of more than two. All variables initially entered into the global model are listed; those that were excluded during model selection are indicated by "Excluded from model".

Appendix Table 1: Sampling details for each site showing the total number of visits, the specific years each site was visited for estimating host abundance indices (deer, sheep, rodents, and birds), and the

929 mean *A. phagocytophilum* prevalence and hazard for each site visit. Data on *Ixodes ricinus* densities

930 and *A. phagocytophilum* prevalence and hazard were gathered during each of the site visits.

931 **Table 1**

Site	*A. phagocytophilum* prevalence per visit (%)	*A. phagocytophilum* hazard per visit (km^{-2})	Deer abundance index per year	Bird abundance index	Rodent abundance index
BM1	5.5	59.0	0.18	7	13.55
BM2	3.6	2.9	0.10	9	
BM3	2.4	12.1	0.75		
BM4	0.5	2.5	0.02	7	8.41
BM5	0.0	0.0	0.00		
BM6	2.1	10.7	0.18	10	8.56
BM7	1.7	1.7	0.20		
BM8	4.2	9.0	0.28	4	3.56
H1	0.0	0.0	0.15		
H2	2.1	0.8	0.08		0.00
H3	4.8	1.5	0.30		
H4	3.5	35.7	1.14		5.49
H5	22.1	45.2	1.90		
H6	0.0	0.0	0.25		5.10
H7	1.3	0.5	0.26		1.04
H8	9.6	2.4	0.50		
PM1	12.5	4.7	0.60		
PM2	1.2	0.4	0.55		
PM3	6.3	33.5	0.97	1	8.80
PM4	7.3	9.4	3.07	1	4.18
PM5	0.0	0.0	0.05		
PM6	11.4	35.1	0.54	0	
PM7	4.0	4.8	0.09	2	12.75
PM8	5.2	21.9	1.00	1	6.93
PY1	3.5	1.7	0.79		4.26
PY2	2.9	2.1	1.79		1.05
PY3	9.3	0.6	0.19		1.08
PY4	10.0	3.1	0.28		1.04
PY5	0.0	0.0	1.95		
PY6	10.6	8.3	4.90		
PY7	10.0	2.2	3.15		
PY8	5.1	3.1	1.85		
SM1	11.0	16.9	2.00		7.37
SM2	9.5	32.9	5.06		2.04
SM3	1.1	1.6	0.25		
SM4	0.0	0.0	0.11		10.31
SM5	4.8	1.0	0.83		2.02
SM6	3.6	4.6	0.25		

SM7	0.0	0.0	0.20		
SM8	0.0	0.0	0.10		

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Table 2

Selected focal model	Estimates (logit)	Std error	p-value	z-value	Δ AICc
deer and sheep (40 sites)					
Intercept	-3.64	0.19	<0.001	-18.97	
Deer abundance index	0.36	0.10	<0.001	3.14	9.2
Sheep presence	Excluded from model				
Habitat	Excluded from model				
Season	Excluded from model				
Birds (10 sites)					
Intercept	-2.63	0.23	<0.001	-11.22	
Bird abundance index	-0.14	0.04	0.001	-3.22	4.8
Deer abundance index	Excluded from model				
Season	Excluded from model				
Rodents (20 sites)					
Intercept	-3.69	0.22	< 0.001	-17.15	
Rodent abundance index	Excluded from model				
Deer abundance index	0.35	0.12	0.003	2.97	5.4
Season	Excluded from model				

937 **Table 3**

Selected models	Estimates (log)	Std error	p-value	z-value	Δ AICc
Deer and sheep (40 sites)					
Intercept	-5.90	0.37	< 0.001	-16.08	
Deer abundance index	0.63	0.20	0.002	3.11	6.9
Ground vegetation	Excluded from model				
Sheep presence	Excluded from model				
Temperature	Excluded from model				
Habitat	Excluded from model				
Season:					19.3
mid summer	-1.01	0.29	<0.001	-3.53	
late summer	-1.45	0.26	<0.001	-5.57	
Birds (10 sites)					
Intercept	-2.35	0.94	0.01	-2.47	
Bird abundance index	-0.11	0.09	0.22	-1.22	-1.3
Temperature	-0.08	0.04	0.05	-1.94	1.0
Ground vegetation	Excluded from model				
Deer abundance index	Excluded from model				
Season:					9.1
mid summer	-0.52	0.36	0.14	-1.46	
late summer	-1.22	0.28	<0.001	-4.42	
Rodents (20 sites)					
Intercept	-7.20	0.59	<0.001	-12.20	
Rodent abundance index	0.24	0.06	<0.001	3.63	7.8
Ground vegetation	Excluded from model				
Deer abundance index	0.83	0.21	<0.001	3.93	11.8
Season:					18.1
mid summer	-0.84	0.29	0.003	-2.94	
late summer	-1.24	0.25	<0.001	-5.04	
Temperature	Excluded from model				

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Appendix table 1:

Site	Number of visits for tick surveys	Tick, sheep, and deer survey years	Rodent survey years	Bird survey years
BM1	5	2018, 2019, 2020	2019, 2020	2018
BM2	4	2018, 2019		2018
BM3	3	2018		
BM4	6	2018, 2019, 2020	2019, 2020	2018
BM5	3	2018		
BM6	6	2018, 2019, 2020	2019, 2020	2018
BM7	3	2018		
BM8	6	2018, 2019, 2020	2019, 2020	2018
H1	3	2018		
H2	4	2018, 2020	2020	
H3	3	2018		
H4	4	2018, 2020	2020	
H5	3	2018		
H6	3	2018, 2020	2020	
H7	4	2018, 2020	2020	
H8	3	2018		
PM1	3	2018		
PM2	3	2018		
PM3	6	2018, 2019, 2020	2019, 2020	2018
PM4	6	2018, 2019, 2020	2019, 2020	2018
PM5	3	2018		
PM6	4	2018, 2019		2018
PM7	6	2018, 2019, 2020	2019, 2020	2018
PM8	6	2018, 2019, 2020	2019, 2020	2018
PY1	4	2018, 2020	2020	
PY2	4	2018, 2020	2020	
PY3	4	2018, 2020	2020	
PY4	4	2018, 2020	2020	
PY5	3	2018		
PY6	3	2018		
PY7	3	2018		
PY8	3	2018		

SM1	4	2018, 2020	2020	
SM2	4	2018, 2020	2020	
SM3	3	2018		
SM4	4	2018, 2020	2020	
SM5	4	2018, 2020	2020	
SM6	3	2018		
SM7	3	2018		
SM8	3	2018		

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