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Multiflora rose invasion amplifies prevalence of Lyme disease pathogen, but not necessarily Lyme disease risk

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Abstract

Background: Forests in urban landscapes differ from their rural counterparts in ways that may alter vector-borne disease dynamics. In urban forest fragments, tick-borne pathogen prevalence is not well characterized; mitigating disease risk in densely-populated urban landscapes requires understanding ecological factors that affect pathogen prevalence. We trapped blacklegged tick (*Ixodes scapularis*) nymphs in urban forest fragments on the East Coast of the United States and used multiplex real-time PCR assays to quantify the prevalence of four zoonotic, tick-borne pathogens. We used Bayesian logistic regression and WAIC model selection to understand how vegetation, habitat, and landscape features of urban forests relate to the prevalence of *B. burgdorferi* (the causative agent of Lyme disease) among blacklegged ticks.

Results: In the 258 nymphs tested, we detected *Borrelia burgdorferi* (11.2% of ticks), *Borrelia miyamotoi* (0.8%) and *Anaplasma phagocytophilum* (1.9%), but we did not find *Babesia microti* (0%). Ticks collected from forests invaded by non-native multiflora rose (*Rosa multiflora*) had greater *B. burgdorferi* infection rates (mean = 15.9%) than ticks collected from uninvaded forests (mean = 7.9%). Overall, *B. burgdorferi* prevalence among ticks was positively related to habitat features (e.g. coarse woody debris and total understory cover) favorable for competent reservoir host species.

Conclusions: Understory structure provided by non-native, invasive shrubs appears to aggregate ticks and reservoir hosts, increasing opportunities for pathogen transmission. However, when we consider pathogen prevalence among nymphs in context with relative abundance of questing nymphs, invasive plants do not necessarily increase disease risk. Although pathogen prevalence is greater among ticks in invaded forests, the probability of encountering an infected tick remains greater in uninvaded forests characterized by thick litter layers, sparse understories, and relatively greater questing tick abundance in urban landscapes.

Keywords: Lyme disease, *Borrelia burgdorferi*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Babesia microti*, Invasive species, Urbanization, Forest fragment

Background

Urbanization affects many aspects of vector-borne disease ecology [1]. In the case of tick-borne disease systems such as Lyme disease (caused by *Borrelia burgdorferi*) in forested ecosystems, urbanization alters habitat suitability for vectors (i.e. ticks), vertebrate hosts, and as a result,

pathogens. Human development in the Lyme disease endemic, mid-Atlantic region of the United States reduces overall forest cover and average patch size while increasing the area of edge and impervious surface. Reduced forest patch size, in particular, results in predictable changes to host community composition that increase acarological risk in terms of nymphal infection prevalence and density of infected nymphs [2–5]. Yet in human-dominated landscapes, patch size may have a smaller or perhaps unpredictable influence on host community relative to other effects of urbanization on forested ecosystems.

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How ecological characteristics of urban forest fragments affect acarological risk has not been well explored.

Complex land use histories in human-dominated landscapes form networks of diverse, heterogeneous forest fragments. In the urban mid-Atlantic region, clear-cutting, intensive agriculture, and urban sprawl have created a variety of forest fragment types on a spectrum between remnants of mature (> 100 yr. old) forests and forest fragments that have regrown from fallow agricultural land set aside while surrounding areas were developed [6]. In the latter case, native tree species have competed with and grown alongside non-native species that were part of the agricultural landscape or subsequent development. As a result, regrown urban forest patches have closed canopies of mostly native trees with thick understories composed of non-native, invasive species [7]. These two extremes of urban forest fragment types both face serious ecological problems (e.g. loss of native understory or reduced regeneration), with implications for tick-borne disease risk.

Due to changes to below-ground processes and browsing pressure from high density white-tailed deer (*Odocoileus virginianus*) populations (in Delaware, recent county surveys estimate between 18 and 52 deer/km² [8]), mature forests may have sparse or no woody understories and cannot replace many species of dead or dying trees [9]. Although they maintain a thick litter layer and low soil pH, which may help buffer mature forests from invasion by non-native plants [10], many native woody plants cannot regenerate. The thick litter layer maintained in these forests provides suitable habitat for blacklegged ticks (*Ixodes scapularis*), which are found in greater abundance in mature forests relative to other urbanized forest fragment types [11]. In contrast, forest fragments with significant non-native plant invasion in the understory have high densities of invasive earthworms and very little leaf litter [12–15], which constrains tick abundance [11, 16, 17]. However, the dense understory structure provided by invasive plants may aggregate immature ticks and infective hosts, potentially amplifying acarological risk in invaded forest fragments [18–21].

Recent studies have identified greater pathogen prevalence in ticks and reservoir hosts associated with invasive shrubs [18–20, 22]. However, because leaf litter loss, which constrains tick abundance, is also associated with non-native plant invasion, it is unclear how tick-borne disease risk differs in regrown, invaded forest fragments compared to mature, uninvaded fragments. To contribute to our understanding of tick-borne disease ecology in urbanized landscapes, we designed a study in urban forest fragments with three objectives: (i) characterize *B. burgdorferi* and emerging tick-borne pathogen prevalence among questing ticks; (ii) test for differences in pathogen prevalence between forests invaded by non-native

understory plants and uninvaded forests; and (iii) determine which habitat and landscape features influence pathogen prevalence.

Methods

Study area and tick collection

We collected nymphal *Ixodes scapularis* ticks from April to July, 2013 and 2014, using CO₂-baited traps in forest fragments around New Castle County, Delaware. Although drag-sampling or flagging is more commonly used to capture *I. scapularis*, we used CO₂-baited traps to avoid confounding results from *Rosa multiflora*'s dense structure (Fig. 1). Even thick, canvas cloth becomes snagged on *R. multiflora* thorns, preventing effective sampling of tick habitat. CO₂-baited traps are unbiased by habitat structure [23]. We built traps following the design of Kensinger & Allan [23], by drilling four holes in 6-quart Coleman® coolers and bolting the coolers to plywood squares. We baited traps for 24 h with 1.4 kg of pelleted dry ice and lined the plywood base with doubled-sided carpet tape (3 M, Maplewood, USA).

Forest fragments (6–16 ha) consisted of mixed deciduous hardwood stands and varied in understory woody species composition, particularly in the extent of non-native *R. multiflora* invasion (Fig. 1, Additional file 1: Figure S1). Each year we trapped ticks in eight forest fragments, four of which had understories with 10–58% of total area covered by *R. multiflora* invasion (hereafter: invaded), and four fragments lacked *R. multiflora* invasion (< 1%), (hereafter: uninvaded). Within invaded sites, we captured ticks at four sets of paired traps: one trap within *R. multiflora* cover and its pair 25 m away, not in *R. multiflora*. Paired traps were separated by 25 m to eliminate the possibility that both traps could be attracting the same ticks [24]. In uninvaded sites, we deployed traps at four random points, separated by at least 25 m. We used a total of 64 trap locations over the 2 yr. study, and half of the traps were active on any given trap night. To avoid weather-related impacts on tick questing behavior, we always deployed paired traps together, and baited in equal numbers of invaded and uninvaded fragments on the same nights. We transported all captured ticks to the laboratory live, in individual microcentrifuge tubes, froze them at -80 °C, and later identified them to species and life stage with dichotomous keys [25–27].

Covariate data collection

We identified a set of 25 variables that we expected would influence *B. burgdorferi* infection rates by increasing or decreasing interactions between larval ticks and competent reservoir hosts (Tables 1 and 2). Further detail on field and computational methods used to collect these data were published in [11, 28]. We surveyed understory vegetation characteristics within a 12.5 m



Fig. 1 Photographs of representative forest sites with *R. multiflora* invasion (a) and without *R. multiflora* (b)

radius surrounding each trap location, which included: estimating the percent of ground covered by *R. multiflora*, the percent of ground covered by coarse woody debris, and the density of understory vegetation using a 2.0 m high Nudds board for which observers estimated

the percentage of each of four 0.5 m panels obscured by vegetation from a distance of 12.5 m [29]. We chose a 12.5 m radius to correspond with the approximate home range size of *Peromyscus leucopus* (an important reservoir for *B. burgdorferi*), while avoiding overlap with

Table 1 Summary of vegetation and landscape covariates measured at the trap scale by forest type and location, modified from [11]. Covariates are summarized as mean \pm standard error. Different superscript letters A, B, C denote significant differences among groups ($P < 0.05$) detected using analysis of variance (ANOVA), blocking on site, followed up with Tukey's *post-hoc* comparisons when there were more than two groups

Covariates	Uninvaded forests	Invaded: in rose	Invaded: not in rose
Trap-level covariates			
Nudds at 0.5–1.0 m (%)	18.0 \pm 3.9 ^A	73.9 \pm 4.5 ^B	53.3 \pm 5.9 ^C
Rose cover, 12.5 m radius (%)	2.5 \pm 0.2 ^A	11.2 \pm 0.6 ^B	7.0 \pm 0.6 ^C
Leaf litter volume (l/m ²)	28.0 \pm 2.8 ^A	6.1 \pm 1.4 ^B	6.7 \pm 1.2 ^B
Coarse woody debris (%)	6.5 \pm 0.9 ^A	3.4 \pm 0.7 ^B	4.2 \pm 0.7 ^B
Rose cover, 2.5 m radius (%)	0.0 \pm 0.0 ^A	67.1 \pm 2.2 ^B	3.5 \pm 0.7 ^A
Distance to agriculture (m)	288.3 \pm 54.7 ^A	156.7 \pm 24.6 ^B	159.6 \pm 24.6 ^B
Distance to edge (m)	67.8 \pm 9.1 ^A	39.8 \pm 8.8 ^B	41.9 \pm 8.4 ^B
Distance to road (m)	154.7 \pm 16.7	135 \pm 18.1	133.4 \pm 15.4
Distance to residential (m)	716.9 \pm 377.6	186.2 \pm 31.5	174.4 \pm 32.9
Distance to stream (m)	371.8 \pm 62.7 ^A	148.4 \pm 35.7 ^B	134.1 \pm 35.6 ^B
Tick abundance ^a	0.8 \pm 0.1 ^A	0.4 \pm 0.1 ^B	0.2 \pm 0.0 ^B
Mouse abundance ^b	2.7 \pm 0.5	4.5 \pm 0.9	2.1 \pm 0.4
Mean larvae per mouse ^b	0.4 \pm 0.1	0.5 \pm 0.1	0.7 \pm 0.2

^aTick abundance values from traps that caught ticks which could be screened for pathogens [11]

^bMouse abundance and larval burdens on mice from concurrent nest box study (Adalsteinsson et al., unpublished data). For trap-level estimates, we calculated the mean of the mice caught during fall at two nest boxes in closest proximity to the tick trap. Larval burdens are the average number of larvae per mice at either the two closest nest boxes

"Nudds" refers to Nudds board (Nudds [29]) measurements and "dbh" stands for diameter at breast height

Table 2 Summary of vegetation and landscape covariates measured at the patch scale by forest type (invaded or uninvaded), modified from [11]. Covariates are summarized as mean \pm standard error. Superscript letters A, B denote significant differences among groups ($P < 0.05$) detected using analysis of variance (ANOVA), blocking on site

Covariates	Uninvaded forests	Invaded forests
Patch-level covariates		
Rose cover (%)	0.8 \pm 0.5 ^A	36.9 \pm 7.7 ^B
Total understory cover (%)	19.6 \pm 4.4 ^A	41.6 \pm 6.1 ^B
Leaf litter volume (l/m ²)	13.9 \pm 1.1 ^A	6.8 \pm 0.9 ^B
<i>Fagus grandifolia</i> (%)	8.5 \pm 2.8 ^A	0.7 \pm 0.2 ^B
<i>Acer</i> spp. (%)	0.7 \pm 0.1 ^A	21.2 \pm 1.2 ^B
Year of canopy closure	1916.7 \pm 4.9 ^A	1963 \pm 5.1 ^B
Non-native stems (%)	9.1 \pm 2.7 ^A	40.0 \pm 3.3 ^B
Average tree dbh (m)	0.6 \pm 0.0	0.6 \pm 0.0
<i>Quercus</i> spp. (%)	42.0 \pm 6.4 ^A	11.0 \pm 5.8 ^B
Mean mice per nest box ^a	0.4 \pm 0.1	0.5 \pm 0.2
Mean larvae per mouse ^a	0.7 \pm 0.2	0.9 \pm 0.3
Bird territory density ^b	3.6 \pm 0.5	5.3 \pm 0.8

^aMouse abundance and larval burdens on mice from concurrent nest box study (Adalsteinsson et al., unpublished data). Total mouse captures and larvae on mice during fall were averaged across all 15 nest boxes in the site

^bSpot mapping data for all ground-foraging bird species, collected during 2010 and 2011 breeding seasons [28]

"dbh" stands for diameter at breast height

paired traps [30]. We also estimated the percent of ground covered by *R. multiflora* within a 2.5 m radius of the trap to more directly represent the effective trapping radius for *I. scapularis* [24]. We quantified leaf litter volume for all litter collected within a 0.5 m² quadrat next to each trap.

We measured landscape variables at each trap location in ArcGIS using a 2007 Delaware land use land cover layer [31], focusing on variables that could influence habitat suitability for ticks and/or hosts and that reflected the human-dominated landscape context of the study area [32, 33]: distance to nearest road, stream, agriculture, forest edge and residential development. We also used data from prior [11, 28] and concurrent studies (Adalsteinsson et al., unpublished data) to quantify abundance of ticks, potential hosts, and host-tick interactions in the study area. Tick abundance at the trap-level was the number of *I. scapularis* nymphs captured at a given trap, standardized by effort (number of trap nights). The densities of ground-foraging bird territories in forest fragments were estimated from spot-mapping surveys conducted during two breeding seasons [28]. Concurrent studies of *P. leucopus* abundance and parasitism by immature ticks (Adalsteinsson et al., unpublished data) provided estimates of mouse abundance and parasitism rates at the trap and forest fragment scale. To study *P. leucopus* abundance, we checked 15 nest boxes per forest fragment once per month; for trap-level estimates, mouse

abundance was the mean of the number of mice caught during fall (larval tick season) at the two nest boxes nearest to the trapping location. For patch-level estimates, the number of mice caught at nest boxes in fall was averaged across all 15 nest boxes in a given forest fragment. Larval tick burdens were the mean number of larvae per mouse at either the two closest nest boxes (trap-level) or across all 15 nest boxes (patch-level).

We also included data collected previously to characterize vegetation at the patch level: proportions of *Fagus grandifolia*, *Acer* spp., *Quercus* spp., *Liriodendron tulipifera*, or *Liquidambar styraciflua* as dominant canopy trees; percent of total area covered by *R. multiflora*; mean leaf litter volume measured at 15 locations in the patch; percent of ground covered by understory plants (all spp.); percentage of understory woody stems that were non-native; and year of canopy closure [28].

Pathogen testing

We used a modified version of the DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands) protocol to extract DNA from ticks. Here, we explain the steps in which we deviated from the manufacturer's protocol. First, we used sterile pipette tips to manually crush each *I. scapularis* nymph individually in 20 μ l of Hyclone Dulbecco's phosphate buffer saline solution (Thermo Fisher Scientific, Waltham, USA). Next, we incubated samples with lysis buffer ATL and proteinase K in a 56 °C hot water bath for 3 h. We performed an extra spin step at 13,000 \times rpm to remove trace ethanol after the Buffer AW2 wash. Finally, we modified the last step by eluting our samples twice (50 μ l each time), for a final product of 100 μ l. We checked concentrations of a subset of our samples using a NanoDrop UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, USA) to confirm successful DNA extractions.

We tested ticks for the presence of *Borrelia burgdorferi* (*sensu lato*), *Anaplasma phagocytophilum*, and *Babesia microti* using a previously described multiplex PCR assay [34]. In addition, ticks were also tested for the presence of *Borrelia miyamotoi* in a TaqMan PCR assay using the following primers and probe: F770-5'-ACC TGC AAC CTT CGG ATT C-3'; R771-5'-TGG TTG TAG CTC AGT TGG TAG-3'; P1277-CalRd610-5'-CTT GTA TCG AAC TAC ACC CAT AGC TC-3'-BHQ2.

Data analysis

A sufficient number of ticks tested positive for *Borrelia burgdorferi* to allow statistical analyses; however, infection prevalence was too low for the remaining pathogens to determine patterns related to invasion and other habitat and landscape features. We tested for spatial autocorrelation in *B. burgdorferi* prevalence across forest fragments using a spline correlogram in package *ncf* [35] in R [36].

Sample sizes were uneven because of differences in tick abundance in invaded and uninvaded sites [11], so we used logistic regression models in a Bayesian framework to compare pathogen (i.e. *B. burgdorferi*) prevalence among ticks collected from invaded and uninvaded forest fragments. This approach allowed us to include uncertainty in pathogen prevalence due to varying sample sizes rather than comparing raw proportions. We first tested whether pathogen prevalence differed among treatments (in rose vs not in rose) within invaded forest fragments. Next, we tested whether pathogen prevalence differed between invaded and uninvaded forests. For both of these questions, we used the following model structure:

$$Y_i \sim \text{Binomial}(1, p_i),$$

$$\text{logit}(p_i) = \alpha + \beta * X_i,$$

where Y is the infection status of each individual (i) tick (0 or 1), which has a binomial distribution with probability p . With the logit link, the probability of infection (p) was estimated as a linear function of baseline infection probability (α) plus the effect of being from one of two treatment groups ($\beta * X$), where the variable X indicates whether the sampling site was either in rose or not in rose, or in an invaded forest or not (0 or 1), depending on the question. We specified vague priors for the parameters α and β including a normal distribution, and we used a Hamiltonian Monte Carlo sampler to run the analysis with STAN [37] using packages *rstan* [38] and *rethinking* [39] in R [36]. Using the difference between posterior distributions (89% highest posterior density intervals) of the estimated pathogen prevalence in two groups (in rose or not in rose, and invaded or uninvaded forest), we calculated the probability of infection prevalence in one treatment group being larger than the other, given our data and the specified model [40].

To understand the influence of habitat and landscape factors on *B. burgdorferi* prevalence, we used aggregated logistic regression models in a Bayesian framework [40]:

$$Y_i \sim \text{Binomial}(n, p_i),$$

$$\text{logit}(p_i) = \alpha + \beta_1 * X_{1i} + \beta_2 * X_{2i} + \dots + \beta_z * X_{zi}.$$

Counts of *B. burgdorferi*-positive ticks (Y_i) were aggregated by trap (i), and the number of trials (n) was the number of ticks tested from each trap. We developed multivariate model sets according to variable types (vegetation, habitat, or landscape) and spatial scale (trap or forest fragment). To select between models, we used Watanabe-Akaike Information Criterion (WAIC) [40–42] and converged on several “best models” that included similar combinations of variables (Table 3). We compared the relative influence of individual variables on the performance of the model by systematically dropping variables and comparing changes in WAIC scores and their standard errors [43].

Results

We tested 258 *I. scapularis* nymphs, from which we successfully extracted DNA (determined through NanoDrop and PCR methods). Twenty-nine ticks (11.2%) were positive for *B. burgdorferi*, five (1.9%) were positive for *Anaplasma phagocytophilum*, and two (0.8%) were positive for *Borrelia miyamotoi*. Only one tick was co-infected with *B. burgdorferi* and *A. phagocytophilum*. We did not find any *Babesia microti*-positive ticks. Spatial autocorrelation in *B. burgdorferi* prevalence across forest fragments was not significant at any distance; at 0 m, the correlation coefficient was -0.61 (95% CI: -1.79–0.72). Within invaded forests, 11 of 75 ticks (14.6%) captured within *R. multiflora* and 6 of 32 ticks (18.7%) captured outside of *R. multiflora* were *B. burgdorferi*-positive. Of 151 ticks tested from uninvaded sites, 12 (7.9%) were positive for *B. burgdorferi*. Based on differences between posterior distributions, we estimated that there was a 64% probability that infection prevalence was greater outside of *R. multiflora* patches within invaded sites. However, there was a 97% probability that infection prevalence was higher among nymphs from invaded sites compared to nymphs from uninvaded sites (Fig. 2).

Table 3 WAIC table of best models. Variables in the model set are coarse woody debris (CWD), leaf litter volume (litter), distance to the nearest road (dist. Road), mouse abundance in fall (mice), total understory cover (total cover), and nymphal tick capture rate (tick abundance). Field headings refer to the effective number of parameters (pWAIC), the difference between WAIC estimates for each model and the top-ranked model (Δ WAIC), the Akaike weight (Weight), the standard error of the WAIC estimate (SE), and the standard error of Δ WAIC value (Δ SE)

Model structure	Δ WAIC	pWAIC	Weight	SE	Δ SE
CWD + litter + dist. Road + mice	0.00	5.10	0.37	20.80	NA
CWD + litter + dist. Road	0.80	3.80	0.25	26.70	3.78
CWD + litter + dist. Road + mice + total cover	0.90	5.80	0.24	21.00	28.10
CWD + litter + dist. Road + mice + tick abundance	2.00	6.40	0.14	21.10	1.98
NULL	13.60	1.00	0.00	21.30	8.36

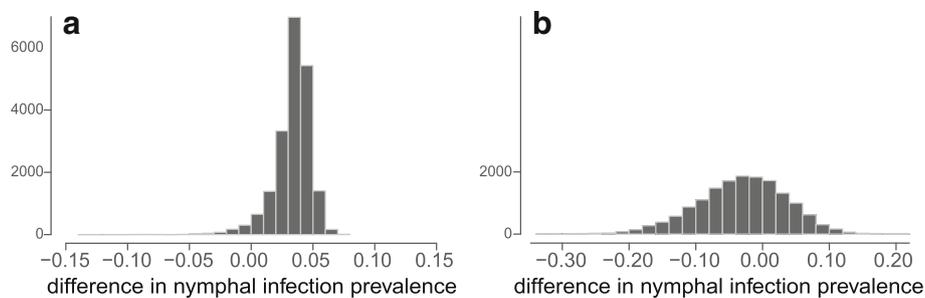


Fig. 2 Distributions of differences between estimated *Borrelia burgdorferi* infection prevalence (posterior distributions) in nymphs between invaded and uninvaded forest fragments (**a**) and within invaded forests, between ticks captured within *Rosa multiflora* and outside of it (**b**). Y-axes display the density of samples from posterior distributions. Between invaded and uninvaded forests (**a**), there is 97% probability that *B. burgdorferi* prevalence is greater in invaded forests. Within invaded forests (**b**), there is only 64% probability that *B. burgdorferi* prevalence is lower within *R. multiflora* stands. Thus, we find support for a difference in *B. burgdorferi* prevalence at the forest-fragment scale (**a**), but not within invaded fragments (**b**)

Among the 50 models tested, the best models for *B. burgdorferi* infection prevalence (Table 3) included woody debris, leaf litter, distance to road, mouse abundance, tick abundance (all at trap-scale), and total cover (fragment-scale). Woody debris, distance to road, mouse abundance, and total cover were positively related to infection prevalence. Leaf litter and tick abundance were negatively related to infection prevalence (Fig. 3).

Discussion

In our comparison of invaded and uninvaded forest fragments, we found that *B. burgdorferi* prevalence among questing ticks did not differ within invaded forests, but that the infection prevalence in ticks from invaded forests was almost double that in ticks from uninvaded forests. *Borrelia burgdorferi* was the most common pathogen detected in nymphal *I. scapularis* from our study sites, followed by *A. phagocytophilum* and *B. miyamotoi*. Only one *I. scapularis* nymph was co-infected with *B. burgdorferi* and *A. phagocytophilum*, and we did not detect *Ba. microti* in any of the ticks tested. At finer scales within both invaded and uninvaded sites, infection prevalence was positively related to coarse woody debris, distance to the nearest road, mouse abundance, and extent of understory cover within the forest fragment. We found a negative relationship between infection prevalence and both leaf litter and tick abundance. *Rosa multiflora* invasion and the additional factors positively influencing pathogen prevalence point to suitable habitat characteristics for small mammal and bird hosts that are competent pathogen reservoirs.

Invaded and uninvaded fragments represent two extremes of different, degraded habitat fragment types that can be separated by the presence/absence of *R. multiflora* invasion in our landscape. Uninvaded sites have deep litter layers, sparse understory, high densities of questing nymphs, and relatively low infection prevalence (mean = 0.079). Invaded sites have very little leaf litter,

dense understory structure, fewer questing nymphs, and roughly double the infection prevalence (mean = 0.159). Our modeling results showed that the total understory cover in a forest fragment positively influences pathogen prevalence. Understory structure, which is provided almost exclusively by invasive plants, may aggregate immature ticks and infective hosts, resulting in increased pathogen prevalence among ticks in invaded forest fragments [19–21]. Because *B. burgdorferi* is not transmitted transovarially [44], infected free-living nymphs acquire the bacteria by feeding on an infected host during their larval stage. Similarly, potential pathogen hosts must acquire *B. burgdorferi* by being fed upon by an infected nymph. Therefore, both immature stages of ticks must interact with infected hosts to elevate pathogen prevalence among nymphs [45].

Understory structure facilitates interactions between immature ticks and competent *B. burgdorferi* reservoir hosts [22, 46, 47], but see [48]. White-footed mouse (*Peromyscus leucopus*) and breeding bird densities are positively correlated with understory structure [47, 49–51] [i.e. invasive plants, in our landscape (unpublished data)]. Within invaded forests, immature ticks are aggregated in stands of invasive shrubs [11, 20, 21]. We hypothesize that larval ticks in uninvaded sites derive a greater proportion of blood meals from larger-bodied hosts that are less-competent *B. burgdorferi* reservoirs [52, 53]. We expect that this is in contrast to larval tick blood meals in invaded sites, which we predict are composed of a greater proportion of small-bodied hosts that are positively affected by understory structure [46] and are competent *B. burgdorferi* reservoirs [53–55]. Future work should use blood meal analysis or identification of *ospC* types in *B. burgdorferi*-positive ticks to understand how non-native plant invasion affects the interaction between specific hosts and ticks, and the resulting implications for transmission of human-invasive *B. burgdorferi* strains [56–58].

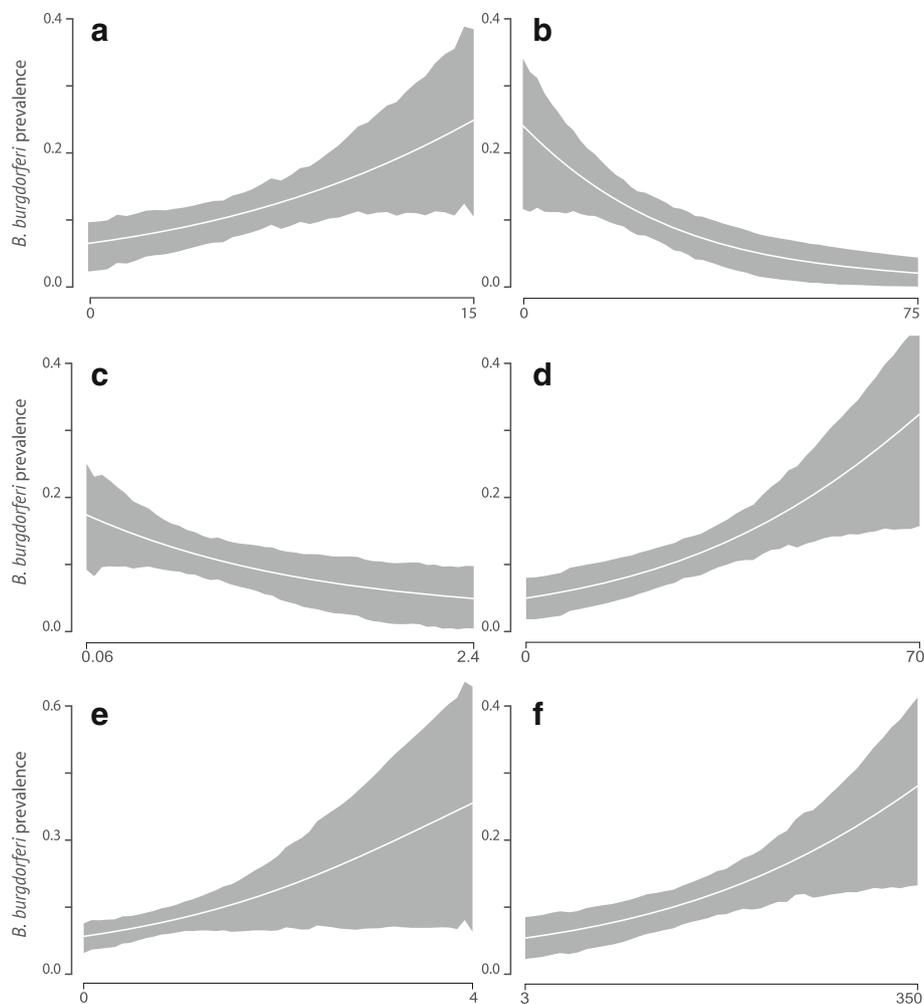


Fig. 3 Mean model-averaged partial predicted responses (with 89% posterior probability intervals) of *Borrelia burgdorferi* prevalence among ticks (proportion of infected ticks) to six different variables: woody debris (%) (a); leaf litter volume (L/m^2) (b); tick abundance (nymphs/24 h) (c); understory cover (%) (d); mouse abundance (mice per nest box per check) (e); distance to nearest road (m) (f). Overall, *B. burgdorferi* prevalence is predicted to increase with increasing woody debris, understory cover, mouse abundance, and distance to nearest road, while increasing leaf litter volume and tick abundance should decrease *B. burgdorferi* prevalence among ticks

An additional hypothesis to explain greater nymphal infection prevalence in invaded sites concerns tick overwinter survival. Invaded habitats lack the litter layer that comprises suitable off-host tick habitat [11, 16, 17]. Ticks depend on the high humidity microclimate within the litter to conserve moisture and to buffer themselves from environmental fluctuations [59]. However, saturated soils coupled with extremely low temperatures may also lead to decreased overwinter survival [60]. Recent studies show that ixodid ticks infected with *B. burgdorferi* have greater energy reserves and are more robust to desiccation [61–64]. Therefore, the harsh litter-free environment of invaded forests may exert stronger pressure against overwinter survival of uninfected ticks, thus increasing overall infection prevalence.

The negative relationships of nymphal infection prevalence with leaf litter and tick abundance raise questions about our understanding of Lyme disease ecology in over-browsed, mature forest fragments. Uninvaded, mature forest fragments that lack understory structure have greater litter volumes and questing tick abundance than invaded forests. We hypothesize that the lack of understory structure in uninvaded fragments shifts the composition of blood meal hosts toward reservoir-incompetent species such as white-tailed deer or other large-bodied hosts [53, 65]. Talleklint & Jaenson [66] also detected a negative relationship between tick density and infection prevalence at high tick densities (> 20 nymphs/ m^2), which they attributed to greater roe deer (*Capreolus capreolus*) densities. Elevated deer densities could account for both

greater tick density and lesser infection prevalence if deer act as both reproductive hosts and the dominant blood meal source [66, 67]. The close proximity among our study sites suggests that deer do not account for differences in tick abundance; most sites are close enough to be within a single deer's home range [68–70] (Additional file 1: Figure S1). However, deer may reduce infection prevalence by shifting blood meals away from reservoir competent hosts that do not find suitable understory cover in over-browsed, uninvaded fragments.

The importance of invasion, habitat, and landscape variables from our models suggest that understory structure and woody debris aggregate infectious hosts and larval ticks, increasing pathogen transmission. Coarse woody debris, total understory cover, distance to road, and white-footed mouse abundance, variables that directly or indirectly represent the distribution of reservoir hosts, were positively related to infection prevalence. Coarse woody debris provides cover, nest sites, movement corridors, and foraging opportunities for immature tick hosts such as white-footed mice, *Sorex* and *Blarina* shrews, and ground-foraging birds [71–75]. Shrews, in particular, are often overlooked in terms of their importance in the Lyme disease system, despite evidence that they can feed and infect more ticks than white-footed mice [76]. Outside of the Pacific Northwest and southern Appalachian regions of the USA, there is a dearth of studies on habitat associations of shrews [74]; in regions where shrews have been well studied, coarse woody debris appears to be an important habitat component [77–80]. Similarly, total understory cover represents the structure available to white-footed mice and shrub-nesting birds [47, 49, 50]. The importance of distance to road suggests that perhaps small mammals and birds avoid hard edges near roads in our landscape, or at least that larval ticks encounter infectious hosts farther from roads.

Conclusions

Although nymphal infection prevalence was greater in invaded forests, acarological risk in terms of density of infected nymphs may be higher in uninvaded sites; the uninvaded sites examined in this study supported ~3 times as many questing nymphs compared to invaded sites [11]. Although uninvaded sites lack understory structure and therefore support lower densities of immature tick hosts, their relatively intact litter layers may allow nymphal ticks to survive longer [81] and quest more often [82], creating more opportunities to attach to humans than in invaded forests. Perhaps in uninvaded fragments, restoration of native understory structure [83] that promotes greater host diversity could reduce densities of questing infected nymphs.

Additional files

Additional file 1: Figure S1. Reproduced without modifications from Adalsteinsson et al. *Ecosphere*. 2016;7(3):e01317 [11] under a Creative Commons license (CC BY 3.0). Map of study area in New Castle County, Delaware. Forest cover is green; agriculture is pale yellow; blue is water; and human development is white. Fragments designated as “rose-invaded” and “uninvaded” refer to the presence or absence of *Rosa multiflora* invasion (TIFF 7506 kb)

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

SAA, WGS, JJB, VD and DB designed the study. SAA trapped ticks, collected field data, and extracted DNA from ticks. JLB obtained permits to collect vertebrate data. WGS, JJB and VD contributed habitat and landscape data. AH performed PCR assays on DNA samples from the ticks. SAA analyzed the data. SAA, WGS and JJB wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Data on white-footed mice was collected under Delaware State Scientific Collecting Permit #2013-007 W and with approval from University of Delaware's Institutional Animal Care and Use Committee under protocol #1249, both of which were issued to JLB.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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