The abundance of *Ixodes ricinus* ticks depends on tree species composition and shrub cover

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SUMMARY

The mainstream forestry policy in many European countries is to convert coniferous plantations into (semi-natural) deciduous woodlands. However, woodlands are the main habitat for *Ixodes ricinus* ticks. Therefore, assessing to what extent tick abundance and infection with *Borrelia* spirochetes are affected by forest composition and structure is a prerequisite for effective prevention of Lyme borreliosis. We selected a total of 25 pine and oak stands, both with and without an abundant shrub layer, in northern Belgium and estimated tick abundance between April and October 2008–2010. Additionally, the presence of deer beds was used as an indicator of relative deer habitat use. *Borrelia* infections in questing nymphs were determined by polymerase chain reactions. The abundance of larvae, nymphs, and adults was higher in oak stands compared to pine stands and increased with increasing shrub cover, most likely due to differences in habitat use by the ticks' main hosts. Whereas tick abundance was markedly higher in structure-rich oak stands compared to homogeneous pine stands, the *Borrelia* infection rates in nymphs did not differ significantly. Our results indicate that conversion towards structure-rich deciduous forests might create more suitable tick habitats, but we were unable to detect an effect on the infection rate.

Key words: *Ixodes ricinus*, *Borrelia burgdorferi*, tick, roe deer, habitat, forest conversion

INTRODUCTION

In recent decades, Lyme borreliosis has become a subject of international concern because of the increasing number of human cases diagnosed each year (World Health Organization, 2004; Bacon *et al*. 2008). The disease is caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex, which is maintained in an enzootic cycle involving mainly ticks of the *Ixodes ricinus* species complex (Acari: Ixodidae) and numerous vertebrates (Piesman and Gern, 2004). Several bird and small mammal species, rodents in particular, serve as hosts for the larval and nymphal stages and are important reservoirs for *Borrelia* spirochetes (Kurtenbach *et al*. 1998; Humair *et al*. 1999; Comstedt *et al*. 2006). Ticks may acquire the spirochetes by feeding on infected hosts, maintain the infection to the subsequent life stages through transstadial transmission, and transmit the infection to other hosts or humans during a next blood meal.

Risk of human infection is typically associated with forested areas, as forests provide ticks with access to a broad range of vertebrate hosts and favourable environmental conditions for tick survival (Gray, 1998). It is commonly assumed that the observed increase in Lyme borreliosis incidence is due to an actual rise in the number of infections and not only to an enhanced surveillance and awareness of the disease. This has been attributed to various factors, particularly to human encroachment into forested areas and habitat modifications, resulting in a closer human contact with ticks and an increase in the abundance and range of tick populations. For instance, the marked increase of deer populations throughout Europe and North America during the latter half of the twentieth century (Gill, 1990; Cederlund *et al*. 1998), largely caused by changes in land use and land cover (e.g., reforestation), has been ascribed a major role in the emergence and spread of Lyme borreliosis (Spielman, 1994; Sood *et al*. 2011). Although incompetent reservoirs for *B. burgdorferi*, deer are the preferred hosts of adult *Ixodes* ticks and play an important role in their reproductive success. Moreover, both tick abundance and the infection prevalence in ticks are favoured by forest fragmentation (Allan *et al*. 2003; Brownstein *et al*. 2005; Halos *et al*. 2010; Tack *et al*. 2012), since

62 deer and rodents benefit from the presence of forest edge habitat and abundant ecotonal vegetation
63 (Tufto *et al*. 1996; Saïd and Servanty, 2005; Boyard *et al*. 2008). In Sweden, ***I. ricinus*** has become
64 more abundant and has expanded its range during the last three decades, which is probably caused
65 by changes in climate (increased duration of the vegetation period and milder winters) and
66 increased abundances of deer (Jaenson *et al*. 2012).

67 In many European countries, the conversion of monospecific coniferous forests into mixed,
68 structure-rich forests dominated by native broadleaved species has become a major objective of
69 sustainable, multipurpose forest management, with the aim of optimising the production of various
70 goods and ecosystem services (Olsthoorn *et al*. 1999; Spiecker *et al*. 2004). However, deciduous
71 forests, especially those harbouring significant numbers of cervids, are generally considered to be
72 ideal habitats for *Ixodes ricinus*, which is the most common tick species associated with Lyme
73 borreliosis in Europe (Gray, 1998). By altering the forest composition and structure, these forest
74 management activities involve a large scale land-use change that might influence the suitability of
75 forests for ticks and, consequently, might influence the epidemiology of tick-borne diseases (i.e.,
76 forest conversion creating an ecosystem dysfunction). Yet, there have been relatively few studies
77 addressing the variation in tick abundance between forest types. While it has recently been
78 quantitatively shown that the abundance of ***I. ricinus*** ticks is higher in oak stands compared to pine
79 stands and increases with increasing shrub cover (Tack *et al*. 2012), little is known on the longer-
80 term temporal variation and on the effects of forest composition and structure on the resulting
81 *Borrelia* infection rate. Here, we selected a total of 25 pine (*Pinus sp.*) and oak (*Quercus sp.*)
82 stands, both with and without a substantial shrub layer, and sampled *Ixodes ricinus* tick populations
83 between April and October in three successive years in northern Belgium to describe the
84 spatiotemporal variation in the abundance of larvae, nymphs, and adults and to relate this variation
85 to forest composition and structure. Additionally, habitat use by cervids was determined by
86 counting the number of deer beds. *Borrelia burgdorferi* s.l. spirochete infections in tick nymphs, the
87 life stage predominantly responsible for pathogen transmission, were determined by polymerase

chain reactions to assess the potential impact of forest conversion on the infection prevalence.

MATERIALS AND METHODS

Study area

The study was conducted at two forest sites in the Campine ecoregion in northern Belgium. Forest site A (51°17' N, 5°12' E) was located near the border with the Netherlands in the municipality Postel and forest site B (51°2' N, 4°58' E) was located approximately 30 km to the south in the municipalities Herselt and Tessenderlo. The climate is sub-atlantic: the mean annual precipitation amounts to 799 mm and is evenly distributed throughout the year, with mean monthly precipitation ranging from 53 mm in March to 79 mm in July. The mean annual temperature is 9.0 °C, with minimum and maximum mean monthly temperatures of 1.4 °C and 16.7 °C in January and July, respectively (Royal Meteorological Institute of Belgium, URL http://www.kmi.be/, accessed November 18, 2011). The region's characteristic forests are pine plantations—mainly consisting of Scots pine (*Pinus sylvestris*) and, to a lesser extent, Corsican pine (*P. nigra* subsp. *laricio*)—on nutrient-poor and acidic sandy soils. The pine stands are interspersed with deciduous stands of pedunculate oak (*Quercus robur*), red oak (*Q. rubra*), common beech (*Fagus sylvatica*), silver birch (*Betula pendula*), and downy birch (*B. pubescens*) (Waterinckx and Roelandt, 2001). Most forests were established in the nineteenth and first half of the twentieth century on former heathlands, which once formed an important component of the traditional agricultural system and covered most of the landscape. The then prevailing microclimatic conditions (temperature and moisture) were most likely limiting for tick survival, which is strongly supported by recent studies carried out in heathlands (Estrada-Peña, 2001; Lindström and Jaenson, 2003; Wielinga *et al*. 2006). However, the large-scale afforestation and the subsequent rise in deer populations probably made this region suitable for tick population establishment and survival. Nowadays, the Campine region is known as a hotspot area in Belgium for Lyme borreliosis (Linard *et al*. 2007). Local vertebrate hosts of nymphal and female ticks are large and medium-sized mammals such as roe deer (*Capreolus*

capreolus), red fox (*Vulpes vulpes*), European hare (*Lepus europaeus*), European hedgehog (*Erinaceus europaeus*), least weasel (*Mustela nivalis*), European pole cat (*Mustela putorius*), and red squirrel (*Sciurus vulgaris*). Very common small mammalian hosts for larvae include pygmy shrew (*Sorex minutus*), common shrew (*Sorex araneus*), wood mouse (*Apodemus sylvaticus*), bank vole (*Myodes glareolus*), and field vole (*Microtus agrestis*) (Verkem *et al*. 2003; Tack *et al.* unpublished data).

Forest stand selection

At each forest site, six pine stands and six oak stands were selected on poor, sandy soils with half of the stands having little or no shrub layer (< 15 % shrub layer cover in the 1–7 m height class) and the other half having a well-developed shrub layer (> 50 % cover). An additional oak stand was selected with low shrub cover at forest site B. In summary, ticks were sampled in 25 forest stands (12 in forest site A and 13 in forest site B) and in four distinct forest stand types: pine stands and oak stands, both with and without a substantial shrub layer. The relative contribution of *Pinus* sp. (*P. sylvaticus* or *P. nigra*) or *Quercus* sp. (mainly *Q. robur*) to the total estimated canopy cover of the tree layer (> 7 m) was greater than or equal to 80 % in each pine and oak stand, respectively. In each forest stand, the percentage cover of the shrub layer (1–7 m) and herb layer (< 1 m) was estimated visually. Shrub cover estimates were very comparable between pine and oak stands at both forest sites. The structure-rich oak stands had an average shrub cover of 66.7 % at site A and 70.0 % at site B, and the pine stands had an average shrub cover of 70.0 % at site A and 58.3 % at site B. The shrub layer mainly consisted of alder buckthorn (*Frangula alnus*), black cherry (*Prunus serotina*), and rowan (*Sorbus aucuparia*) in the pine stands and alder buckthorn, pedunculate oak, and sycamore (*Acer pseudoplatanus*) in the oak stands. The herbaceous layer was dominated either by wavy hair-grass (*Deschampsia flexuosa*), purple moor-grass (*Molinia caerulea*), broad buckler-fern (*Dryopteris dilatata*), or bilberry (*Vaccinium myrtillus*), providing a comparable blanket contact when drag sampling for ticks (see below). Forest stands with a dense bracken (*Pteridium*

aquilinum) understory were avoided because this vegetation can seriously impede tick sampling (Tack *et al*. 2011). Because of the height and rough vegetation surface of bracken, ticks are easily brushed off the blanket, causing tick abundance to be underestimated. However, the sampled vegetation types are representative for the Campine region so we do not expect our sampling procedure to greatly affect the results.

Sampling strategy

Tick sampling was carried out between April and October in 2008, 2009, and 2010 for a total of eleven occasions at site A and twelve occasions at site B (12 stands × 11 occasions + 13 stands × 12 occasions = 288). Sampling consisted of dragging a white flannel blanket (1 × 1 m²) over the herbaceous vegetation and litter. In each forest stand and at each sampling occasion, we performed six one-minute blanket drags (each extending a distance of *ca.* 25 m) at random and recorded the air temperature and relative humidity three times at a height of 1.25 m above the soil surface, using a portable digital temperature and relative air humidity meter (DM509, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). Sampling was always performed on dry (no rain) and non-windy days (< 2 Bft) during day time (between 10:00 am and 05:00 pm) when the vegetation was dry. To avoid time of day and changing meteorological conditions as a source of bias, the four forest stand types were sampled in random order on each sampling day. After each transect, larvae, nymphs, and adults were removed from the blanket using forceps and stored in vials containing 70 % ethanol for later identification and counting. The ticks were counted and identified morphologically with a stereo-microscope using the identification keys of Hillyard (1996).

Additionally, the number of faecal pellet groups and beds of roe deer were counted at each sampling occasion, along the same transects used for tick sampling. Pellet-group counting is a widely used method for assessing habitat use by deer. In our study, however, the number of pellet groups counted was too small (only 21 pellet groups in total) for proper analysis. Instead, we have used the number of deer beds in each forest stand type to examine differences in habitat selection

for bedding sites (Smith *et al*. 1986; Bíró *et al*. 2006). Deer beds were easily detectable in the sandy soil of the study area and were distinguished as oval depressions in the soil or as flattened areas of vegetation, often accompanied by other signs of roe deer (e.g., hoof prints, hair).

Identification of Borrelia infections

Twenty pooled samples per forest site per year (20 samples × 2 sites × 3 years = 120), with each sample consisting of five nymphs, were used for further molecular analyses for the presence of *B. burgdorferi* s.l. spirochetes. We did not identify the *Borrelia* genospecies. Instead, only screening up to species level was performed to get an idea of the overall infection prevalence. For each forest site and each year, ten samples consisted of nymphs collected in pine stands with low shrub cover while the other ten samples were collected in oak stands with high shrub cover. Potential differences in infection prevalence are most likely to occur between these two contrasting forest stand types. DNA was extracted using the method of Boom *et al*. (1990). This method is based on the lysing and nuclease-inactivating properties of proteinase K together with the nucleic acid-binding properties of silica particles. A standard PCR amplification was performed in 25 µL reaction mixtures containing 5 µL of the extracted DNA, 1.65 mM $MgCl_2$, 0.2 mM of all four dNTPs, 10 pM of two primers (BorrSLospAF/BorrSLospAR) (Demaerschalck *et al*. 1995), 1 UTaq polymerase enzyme (Promega), and 1 µL Yellow SubTM (GENEO Bioproducts, Hamburg, Germany). After a hot start of 10 s at 84 °C, an initiation of 4 min at 92 °C was performed, then followed by a 40 cycles denaturation-hybridisation-elongation step (30 s at 92 °C, 45 s at 58 °C, and 60 s at 72 °C). The PCR ended with an extension step of 10 min at 72 °C. Five microlitre of each reaction mixture was mixed with 2 µL of loading buffer and loaded onto 2 % agarose gels (Sigma) to be examined for the presence of DNA fragments. A 1.5 kb DNA ladder (MBI Fermentas, Lithuania) was loaded on every gel. The samples were run for 20 min at 100 V, stained in ethidium bromide for 30 min, washed under running tap water, and photographed under UV illumination.

Statistical analysis

Questing tick abundance, expressed as the number of ticks collected per 100 m², was first $\log_{10}(n+1)$ transformed to approach normality, which was verified using the Kolmogorov-Smirnov test. Subsequently, log-transformed tick abundances were modelled with linear mixed models using the *lmer*-function of the *lme4*-library (Bates *et al*. 2011) in R 2.13.0 (R Development Core Team, 2011). Data for each life stage (larva, nymph, and adult) were analysed separately. Models included tree species (pine vs. oak), shrub cover (in %), herb cover (in %), year, and all their two-way interactions as fixed effects and forest stand (nested within forest site (A or B)) and sampling occasion as non-nested random effect terms. To analyse the effects of tree species, shrub cover, year, and all their two-way interactions on the presence of roe deer (scored as 1 or 0 depending on whether deer beds were (1) or weren't (0) encountered in the forest stand while dragging), we applied a generalised linear mixed model (GLMM) with similar random-effects structure as above, but with a binomial error distribution and logit link function. Analysis of nymphal infection with *B. burgdorferi* s.l. (pooled samples of nymphs infected (1) or not (0)) were also performed with a GLMM with binomial error distribution and logit link function. This model included forest type (pine stands with low shrub cover vs. oak stands with high shrub cover), year, and their interaction term as fixed effects and forest stand (nested within forest site) as random effect term. We always compared all possible models (i.e., build by each combination of the fixed effects terms) using Akaike's Information Criterion, adjusted for sample size (AIC_C) (Hurvich and Tsai, 1989). The ΔAIC_C of a model was then calculated as the difference in AIC_C value for that model and the model with the lowest AIC_C value (best fit to the data). Models with $\Delta AIC_C \leq 4$ were considered equivalent (Bolker, 2008). To determine the relative importance of the explanatory variables, we used the sum of Akaike weights of the set of all top models ($\Delta AIC_C \leq 4$) in which the variable appeared (Burnham and Anderson, 2002). The Akaike weight reflects the weight of evidence in support of a particular model relative to the entire model set, and varies from 0 (no support) to 1 (complete support). Finally, the parameter values of the model with the lowest AIC_C value were estimated with restricted maximum likelihood estimation.

RESULTS

A total of 110,770 *I. ricinus* ticks were collected, of which 89,017 were larvae, 18,685 were nymphs, and 3068 were adults (1634 males and 1434 females). During tick collection, the air temperature ranged from 7.1 °C to 31.7 °C and the relative humidity ranged from 28.1 % to 92.6 %. The mean ± standard error of the number of ticks collected per 100 m² was 206.1 ± 20.8 larvae (range 0–4263), 43.3 ± 2.1 nymphs (range 1–215), and 7.1 ± 0.4 adults (range 0–44). On each sampling occasion, all three life stages were active and ticks were found questing in all 25 forest stands studied. In May 2009, a very high number of larvae was collected along a single transect in one of the oak stands with high shrub cover, which resulted in a peak in larval activity in May (Fig. 1a). This high variance in larval abundance was not unexpected and reflects the limited dispersal capability of larvae after emergence from the egg mass, consisting of up to 2000 eggs (Jongejan, 2001). By considering this single transect as outlier, questing larvae showed a summer peak (August) each year. Nymphs were active throughout the study period without displaying a clear peak (Fig. 1b). Adult tick abundance peaked in spring (April–May) each year and steadily declined in summer (Fig. 1c). Our data were not suited to study seasonal variation in tick abundance, but our results are in line with those of Gassner *et al*. (2011), who examined the temporal dynamics of *I. ricinus* in a neighbouring country, the Netherlands.

For both larvae and adults, the best model explaining the variation in tick abundance included tree species and shrub cover as explanatory variables (Table 1). For adults, a second closely competing model also included a tree species by year interaction term. The best model for nymphs included tree species, shrub cover, and year, whereas the second best model included only tree species and year (Table 1). Herb cover did not appear in any of the top models. Tree species, on the other hand, was present in all top models of each life stage and was therefore the variable with the highest relative importance in explaining tick abundance (Table 2). The temporal fluctuations in tick abundance were very similar in oak and pine stands, but the mean abundance was consistently

239 higher in the oak stands (Fig. 1a–c; Table 3). Larvae, nymphs, and adults were on average 3.3, 1.6,
240 and 1.5 times more abundant in the oak stands. Shrub cover was also a variable of high relative
241 importance (Table 2) and had a positive effect on tick abundance (Table 3). On each sampling
242 occasion, the mean number of ticks collected was higher in forest stands with high shrub cover
243 compared to stands with low shrub cover. Overall, the number of larvae, nymphs, and adults was
244 2.1, 1.5, and 1.8 times higher in forest stands with high shrub cover (> 50 % cover) (Fig. 2a–c).
245 Hence, mean tick abundance was lowest in pine stands with low shrub cover (43.3 ± 8.2 larvae,
246 20.7 ± 2.0 nymphs, and 3.8 ± 0.5 adults per 100 m²) and highest in oak stands with high shrub cover
247 (418.9 ± 72.2 larvae, 61.6 ± 4.9 nymphs, and 11.1 ± 1.0 adults per 100 m²) (Fig. 2a–c).

248 A very similar pattern was observed regarding the number of deer beds we encountered during tick
249 sampling (Fig. 1d; Fig. 2d). The best model explaining the presence of deer beds included tree
250 species, shrub cover, and year (Table 1), with the first two being the variables with the highest
251 relative importance (Table 2). The probability of encountering deer beds was significantly higher in
252 oak stands ($n = 288$, $p = 0.006$) and in forest stands with high shrub cover ($n = 288$, $p = 0.015$)
253 (Table 3). The mean number of deer beds was 1.6 times higher in forest stands with high shrub
254 cover and twice as high in oak stands, which resulted in four times as many deer beds in oak stands
255 with high shrub cover compared to pine stands with low shrub cover.

256 *Borrelia*-positive nymphs were found each year at both forest sites. The average infection rate with
257 *B. burgdorferi* s.l. was 8.3 % (95 % confidence interval: 4.8–13.2 %) in 2008, 11.3 % (7.0–16.9 %)
258 in 2009, and 6.2 % (3.4–10.7 %) in 2010. A similar infection rate was observed at both forest sites
259 in the first two years of our study, but the infection rate at site A (1.0 %) was considerably lower
260 compared to site B in 2010 (12.9 %). No significant difference in infection rate was observed
261 between the homogeneous pine stands and the structure-rich oak stands ($n = 120$, $p = 0.850$). The
262 average infection rate was 8.3 % (5.4–12.2 %) in the pine stands and 8.7 % (5.7–12.8 %) in the oak
263 stands.

DISCUSSION

Our results show that tree species composition and vertical structure are important variables in explaining tick abundance in forests. The abundance of all three life stages was higher in oak stands compared to pine stands, and increased with increasing shrub cover. Interestingly, this pattern was observed at both forest sites and on almost every sampling occasion. So, although some annual and seasonal fluctuation in tick numbers occurred, the mean tick abundance was always lowest in the homogeneous pine stands and almost always highest in the structure-rich oak stands. On average, the abundance of larvae, nymphs, and adults was 9.7, 3.0 and 2.9 times higher in the oak stands with high shrub cover than in the pine stands with no or little shrub cover, while intermediate abundances were recorded in the two remaining forest stand types. The observed differences in tick abundance between the forest stand types must not necessarily depend directly on differences in tree species composition or structure, but may rather be caused by differences in activity of host animals. Our observations from deer bed counts indicate that roe deer were more often present in oak stands and in stands with high shrub cover, most likely because of the availability of high-quality forage and shelter. The importance of deer in maintaining tick populations has been stressed in several European studies (Gray *et al*. 1992; Pichon *et al*. 1999; Ruiz-Fons and Gilbert, 2010). Being the most common large mammals in the study area, roe deer are almost certainly the most important hosts for adult ticks and, therefore, their habitat use largely determines the location where engorged female ticks drop off and lay eggs. The immature stages (larvae and nymphs) also feed on large mammals such as roe deer, but they generally feed on small to medium-sized mammals and birds. Rodents, such as bank vole and wood mouse, have been identified by several authors as key hosts for larval ticks (Tälleklint and Jaenson, 1997; Humair *et al*. 1999; Estrada-Peña *et al*. 2005). These rodent species, together with other mammal species such as foxes and hedgehogs, are common in the study area and provide immature ticks the opportunity to successfully obtain a blood meal and develop into the next life stage, which explains the relatively high nymphal and adult abundances in our study.

290 Besides being important hosts for immature ticks, small mammals and birds are also important
291 reservoir hosts for *Borrelia* spirochetes. *Borrelia afzelii* has been associated with mice, voles, and
292 red squirrels, *B. burgdorferi* sensu stricto with red squirrels, and *B. garinii* and *B. valaisiana* mainly
293 with birds (Humair and Gern, 1998; Kurtenbach *et al*. 1998; Humair *et al*. 1999; Hanincová *et al*.
294 2003). The different genospecies tend to cause distinct clinical manifestations affecting different
295 systems (van Dam *et al*. 1993) and, thus, the vertebrate host composition will determine not only
296 the density of *Borrelia* infected ticks but also the relative risk of different clinical forms of Lyme
297 borreliosis. We did not identify the *Borrelia* genospecies, which could be considered a shortcoming
298 of our study. However, *B. afzelii* and *B. garinii*, both known to be pathogenic to humans, are the
299 two most common *Borrelia* species in Belgium, the Netherlands, and northern France (Rauter and
300 Hartung, 2005), suggesting that most larvae feed on small rodents and birds in this region. A study
301 carried out in the Netherlands (Gassner *et al*. 2008) showed a significantly higher nymphal
302 abundance and *Borrelia* infection rate in oak plots than in pine plots, which was ascribed to
303 differences in rodent densities. In our study, however, the nymphal infection rate with *Borrelia*
304 varied substantially for the different forest sites and years, but no significant effect was found for
305 forest type. Yet, as the absolute number of ticks was considerably higher in oak stands and stands
306 with an abundant shrub layer, the chance of getting bitten by ticks and acquiring infection is in fact
307 influenced by forest type.

308 The results of this study have important implications for forest management, as management
309 activities can alter the composition and structure of forests, which could have a profound impact on
310 the epidemiology of tick-borne diseases such as Lyme borreliosis. In response to environmental
311 concerns and changing societal needs, one of the main goals of the forest management policy in
312 many parts of Europe is the conversion of (often coniferous) plantations to semi-natural forest
313 types. To achieve this, large areas of homogeneous coniferous stands are being converted into
314 mixed, structure-rich deciduous stands with oak as one of the main constituents. Our results indicate
315 that this forest type can support higher tick population levels than monospecific plantations.

However, whereas tick abundance was highly affected by tree species and shrub cover, the overall *Borrelia* infection rates in ticks were similar in the two contrasting forest types. On the other hand, it is important to note that monospecific pine stands cover most of the area in both forest sites, while oak stands, especially those with an abundant shrub layer, are relatively scarce. Large-scale forest conversion programs could change the composition and abundance of wildlife communities to the extent that the relative proportion of reservoir-competent and incompetent hosts changes, thereby influencing not only tick abundance but the infection prevalence in ticks as well. In the past decade, increasing attention has been paid to the role of biodiversity in mediating infection levels and disease, termed the dilution effect (Ostfeld and LoGiudice, 2003). The current study underlines the importance of considering spatial heterogeneity in forest habitat quality when studying tick populations and supports vegetation management as a tool to control tick populations. Relatively simple interventions such as mowing the vegetation and clearing brush along forest trails have been shown to be effective in reducing the local abundance of ticks (Wilson, 1986; Schulze *et al*. 1995). However, further studies will be required in order to fully understand the effects of forest conversion on Lyme borreliosis risk.

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Table 1. Model selection statistics for the analyses of effects of tree species (T), shrub layer cover (S), and year (Y) on the abundance of *Ixodes ricinus* larvae, nymphs, and adults and on the presence of deer beds. ΔAIC_C: the difference in values of the corrected Akaike Information Criterion (AIC_C) between a model and the best model having the lowest AIC_C value; *w*: Akaike weight, indicating relative support for the model.

Response	Model	d.f.	ΔAIC_C	*w*
Larvae	T + S	7	-	0.630
	T + S + Y	9	2.31	0.198
	T	6	2.60	0.172
Nymphs	T + S + Y	9	-	0.684
	T + Y	8	1.54	0.316
Adults	T + S	7	-	0.490
	T×Y + S	11	1.17	0.273
	T + S + Y	9	2.75	0.124
	T	6	2.94	0.113
Deer beds	T + S + Y	8	-	0.311
	T + S	6	0.42	0.253
	T×S + Y	9	2.04	0.112
	T×S	7	2.43	0.092
	T×Y + S	10	3.00	0.070
	T + Y	7	3.13	0.065
	T	5	3.55	0.053
	T + S×Y	10	3.89	0.044

503 Table 2. Relative importance of each explanatory variable, calculated across all top models (ΔAIC_C

504 ≤ 4, see Table 1) in which the variable appeared.

Variable	Larvae	Nymphs	Adults	Deer beds
T	1.000	1.000	1.000	1.000
S	0.828	0.684	0.887	0.882
Y	0.198	1.000	0.397	0.602
T×S	0.000	0.000	0.000	0.204
T×Y	0.000	0.000	0.273	0.070
S×Y	0.000	0.000	0.000	0.044

505

506 Table 3. Parameter estimates (P.E.) of the best model (see Table 1) for the abundance of *Ixodes*
507 *ricinus* larvae, nymphs, and adults and for the presence of deer beds. A positive effect for tree
508 species means a higher tick abundance or deer presence in oak stands compared to pine stands. A
509 positive effect for the year 2009 or 2010 means a higher tick abundance or deer presence in that
510 year compared to 2008.

	Larvae		Nymphs		Adults		Deer beds	
Source of variation	P.E.	t-value	P.E.	t-value	P.E.	t-value	P.E.	z-value
Intercept	2.931	8.871	2.330	12.170	1.341	7.270	-0.873	-1.469
Tree species	1.428	5.505	0.604	4.870	0.394	3.589	1.189	2.760
Shrub cover	0.017	4.287	0.008	4.381	0.008	4.725	0.016	2.429
Year 2009			0.588	2.906			-0.594	-0.992
Year 2010			0.873	4.135			-1.450	-2.290

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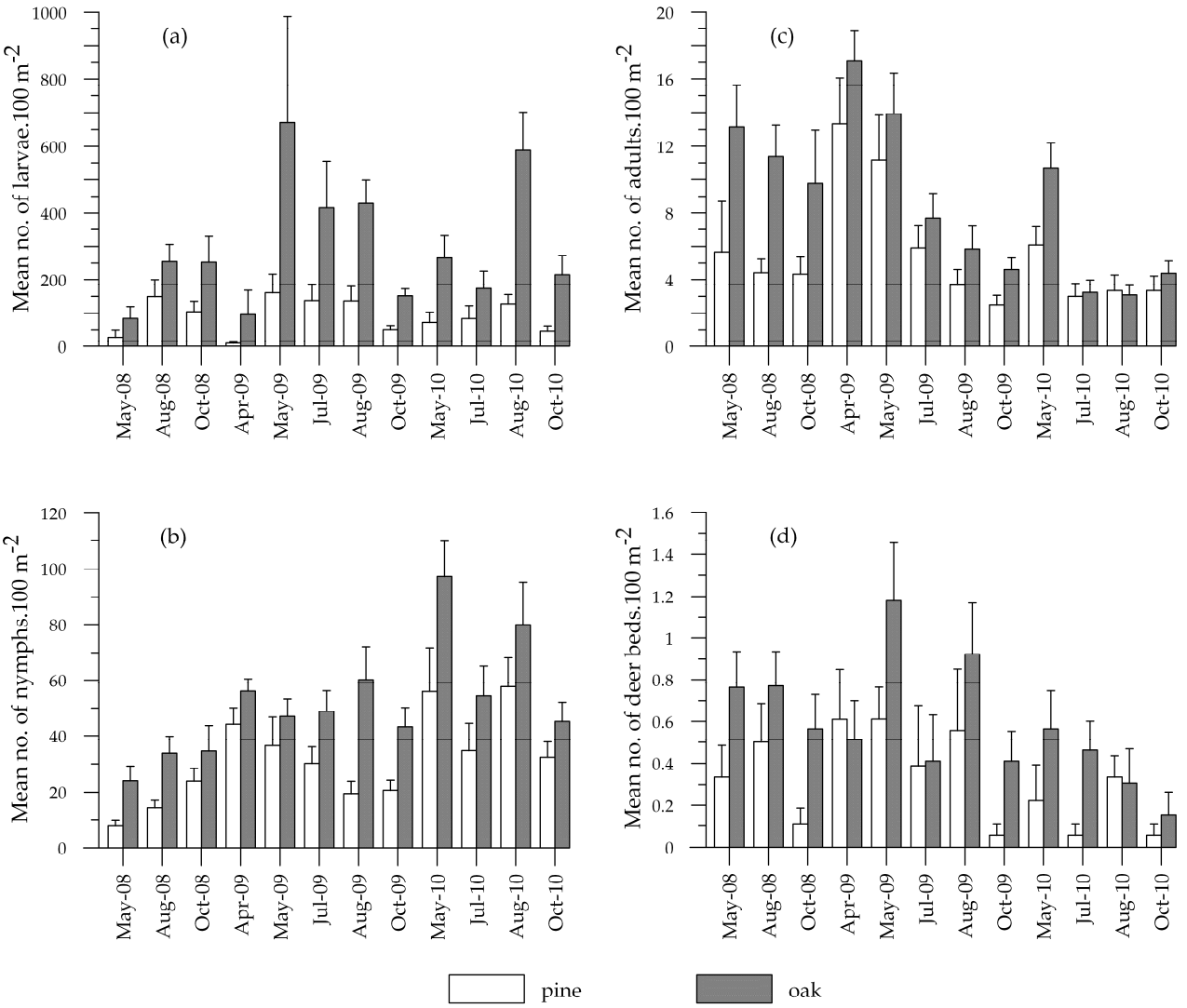
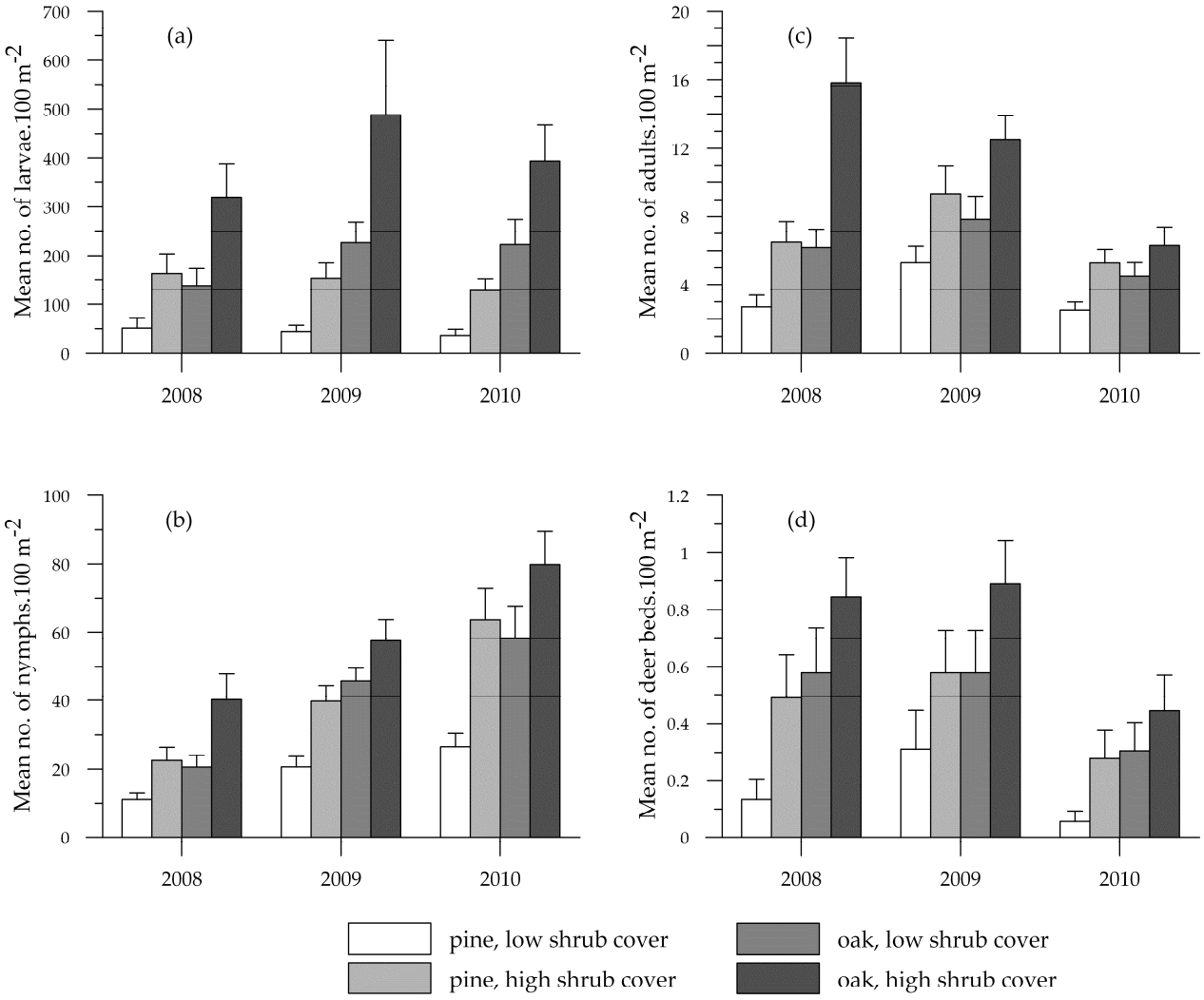


514

515 Fig. 1. Mean number of *Ixodes ricinus* larvae, nymphs, and adults (a–c) and mean number of deer
516 beds (d) in pine and oak stands between May and October in three successive years. The results
517 from the two forest sites were pooled. Error bars denote the standard error of the mean. Note the
518 difference in values on the y-axis.



519

520 Fig. 2. The effects of tree species and shrub layer cover on the number of *Ixodes ricinus* larvae,
521 nymphs, and adults (a–c) and on the number of deer beds (d) in three successive years. Shrub cover
522 estimates were grouped into two classes: low (< 15 %) and high (> 50 %) cover. The results from
523 the two forest sites were pooled. Error bars denote the standard error of the mean. Note the
524 difference in values on the y-axis.