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Cascading impacts of overstory structure in managed forests on understory structure, microclimate conditions, and *Ixodes scapularis* (Acari: Ixodidae) densities

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Forest management practices designed to meet varied landowner objectives affect wildlife habitat and may interrupt the life-cycle stages of disease vectors, including the black-legged tick, *Ixodes scapularis* Say (Acari: Ixodidae). *Ixodes scapularis* transmits multiple pathogens including *Borrelia burgdorferi*, the causative agent of Lyme disease, which is the most common tick-borne disease in the United States. There is evidence that a range of active forest management practices (e.g., invasive plant removal, prescribed burning) can alter tick densities and pathogen transmission. However, few studies have investigated relationships between forest stand structural variables commonly manipulated by timber harvesting and tick ecology. Foresters may harvest timber to create certain forest structural conditions like the mean number of trees, or basal area, per hectare. This study used a spatially replicated experiment in a blocked design to compare forest stands with a range of overstory structures and document variations in the midstory, understory, and forest floor, as well as microclimate conditions within tick off-host habitat. Greater numbers of trees or basal area per hectare correlated with greater canopy closure but less understory cover, stabilized microclimate temperature, higher microclimate humidity, and greater *I. scapularis* nymph densities. A random forest model identified understory forest structure as the strongest predictor of nymph densities. There was no relationship between the number of trees or basal area per hectare and daily deer (*Odocoileus virginianus* Zimmermann) activity or nymphal infection prevalence. These findings provide a deeper understanding of tick-habitat associations within a forest stand and have the potential to inform forest management decisions.

Key words: black-legged tick, timber harvest, silviculture, tick-borne pathogen, *Borrelia burgdorferi*

Introduction

Many hard-bodied ticks (Acari: Ixodidae) and the pathogens they transmit are deeply embedded in forest ecosystems, and numerous studies have documented the impact of forest configuration and management on tick densities and infection prevalence (Allan et al. 2003, Elias et al. 2006, Allan 2009, Williams et al. 2017, Ginsberg et al. 2020, Hodo et al. 2020, Conte et al. 2021, López-Pérez et al. 2021, Brennan et al 2023). Forest management practices shape the environment in which ticks, their vertebrate hosts, and pathogens interact. At the forest stand scale, there is evidence that a range of active forest management practices can alter tick densities and pathogen transmission. For example, in North American forests removal of Amur honeysuckle (*Lonicera maackii* (Rupr.) Maxim) decreased *Amblyomma americanum* Linnaeus abundance (Allan et al. 2010), while the presence of Japanese barberry (*Berberis thunbergia* de Candolle) and other Eurasian honeysuckle species (*Lonicera* spp.) correlated with higher *Ixodes scapularis* Say abundance (Elias et al. 2006) and *Borrelia burgdorferi* infection prevalence (Williams et al. 2009, 2017). Leaf litter removal (Schulze et al. 1995) and prescribed burns (Gleim et al. 2014, Hodo et al. 2020) also decreased *I. scapularis* and *A. americanum* abundance.

Overstory structure, which is often manipulated by timber harvesting, may have similar impacts on ticks, yet little prior research has addressed its relationship to tick densities and pathogen infection prevalence. Timber harvesting is one of the most common forms of active forest management on privately owned land (Butler 2008, Butler et al. 2021), but the least-studied practice in the context of tick-borne disease. In the United States, roughly 39% of forested land is owned by private, non-industrial landowners (Butler et al. 2021) and produces up to 60% of the nation's wood supply (Adams et al. 2006). Forest owners often harvest their timber to realize income, promote regeneration, improve hunting and/or recreation, minimize insect and disease damage, or generate firewood for personal use (Ernst and Knapp 1985, Butler 2008). The few existing studies showed reduced *I. scapularis* densities in stands harvested for timber in the past 5 yr compared to non-harvested stands (Conte et al. 2021). Similarly, other work found reduced questing *Ixodes pacificus* abundance in harvested stands (López-Pérez et al. 2021), and reduced *I. scapularis* abundance in thinned plots versus unthinned plots (Brennan et al. 2023).

The mechanisms driving relationships between timber harvesting, forest structure, and tick abundance may include changes to the microclimate (Ginsberg et al. 2020, Brennan et al. 2023) and vertebrate host communities (López-Pérez et al. 2021). Tick off-host survival and behavior depend heavily on microclimate conditions (e.g., temperature and humidity) of the forest floor (Ogden et al. 2004, Berger et al. 2014), where ticks spend most of their life cycle (Roe and Sonenshine 2014, Burtis et al. 2019). Removing trees and opening the canopy may create drier and warmer microclimates, in turn reducing tick survival or abundance (Conte et al. 2021, Brennan et al. 2023). Additionally, transient declines in small mammal abundance 1-yr post-harvest may result in reduced tick abundance as well, though the long-term effects are unknown (López-Pérez et al. 2021). Reduced small mammal foraging activity in harvested and exposed habitats may also decrease tick-host encounter frequencies, indirectly lowering tick abundance (Conte et al. 2021). Overall, previous work has investigated the effects of timber harvesting and forest structure on tick populations by examining harvested versus unharvested plots and select vegetation variables, and considered microclimate and host mechanisms to explain documented patterns. Yet we still do not know the consequences of a range of structural conditions resulting from various harvesting intensities implemented more than 5 yr prior, nor whether specific forest structures in the midstory and understory in addition to the overstory are related to tick densities. The ecological mechanisms that explain correlations between forest structural attributes directly manipulated by timber harvesting and tick densities require further investigation.

Timber harvesting reduces the number of trees and the basal area per hectare (i.e., the area occupied by tree stems, dependent on both the number and size of individual trees). Removing trees causes structural changes in the overstory with potential consequences for the structure of other forest layers. Little previous research has characterized simultaneously the overstory, midstory, understory, forest floor, and microclimate in relation to tick density and pathogen infection prevalence. While many studies have investigated the influence of habitat structure on tick populations (Elias et al. 2006, Prusinski et al. 2006, Swei et al. 2011, Adalsteinsson et al. 2016, Ginsberg et al. 2020), there has yet to be a comparison of how forest structure variables across all forest layers relate to one another and microclimate variables, especially as a consequence of previous timber harvesting, alongside how any altered structure variables in turn influence tick densities. Numerous forestry variables throughout all forest layers (e.g., number of trees per hectare, basal area per hectare, tree species composition, sapling counts, and leaf litter depth) may affect tick densities. Moreover, there may be interactions between these variables at different scales (i.e., overstory to midstory to understory to forest floor and microclimate), leading to an ecological cascade in which overstory structure manipulated by timber harvesting influences tick-borne pathogen transmission via multiple mechanistic pathways. Such pathways include altered tick densities through changes in tick off-host survival and behavior due to microclimate, and encounter frequencies with key vertebrate hosts, such as white-tailed deer (*Odocoileus virginianus* Zimmermann) and white-footed mice (*Peromyscus leucopus* Rafinesque) (LoGiudice et al. 2003, Keesing et al. 2012). Investigating structure at all forest layers created through timber harvesting betters our basic understanding of the forest structural attributes influencing ticks and vertebrate hosts in the Lyme disease system, with implications for applied forest management practices that promote structural characteristics that mitigate tick densities and/or infection prevalence.

The objectives of this study were to (i) determine the impacts of the number of trees and the basal area per hectare in the overstory of forested stands on density and infection prevalence of *I. scapularis* nymphs in mixed-wood forests in the northeastern United States, and (ii) explore correlations between nymph density and biotic or abiotic factors affected by harvesting. This study focuses on *I. scapularis* nymphs as this life stage is the most epidemiologically significant (Spielman et al. 1985). We conducted the study in a spatially replicated design. We compared tick densities and infection prevalence spanning a range of trees and basal area per hectare, 2 forest inventory metrics widely used by foresters in developing management plans. To understand how these metrics' cascading effects may ultimately alter tick densities and infection prevalence, we examined possible pathways linking forest structure to microclimate conditions and daily white-tailed deer activity, and then to ticks. We developed a random forest model to elucidate the attributes most strongly correlated with *I. scapularis* densities to identify potential drivers of tick densities.

Materials and Methods

Study Area Selection and Study Design

Our 2-yr exploratory study took place from 24 June to 21 August 2019, and 23 June to 27 August 2020, in the Cumberland, Kennebec, and York counties, Maine. These counties have a high incidence of Lyme disease with a combined 943 cases/100,000 people in 2019 (Maine Center for Disease Control and Prevention 2021), which correlates with the geographic distribution of *I. scapularis* in the state (Elias et al. 2020, 2021, Rounsville et al. 2021). Additionally, Maine is 89% forested (USDA Forest Service 2022), has one of the highest harvest rates in the Northeast United States of America (Buchholz et al. 2011, Canham et al. 2013, Brown et al. 2018), and 92% of the forests are privately owned (Bergdahl et al. 2019, USDA Forest Service 2022). Thus, the region is an ideal environmental setting in which to investigate the impacts of forest structural attributes manipulated by timber harvesting on *I. scapularis* ecology.

We conducted a spatially replicated experiment in a blocked design. Five locations, typical of non-industrial private forests in southern Maine, were the blocks (Fig. 1a), each containing 3 replicate stands spaced at least 500 m apart to maintain independence (Fig. 1c). One stand was excluded from data analyses due to wet conditions throughout the season, so the study was conducted on a total of 14 experimental units. Each experimental unit was a 1-ha eastern white pine—mixed hardwood forest stand (Fig. 1c) consisting of 50% or more northern hardwood tree species

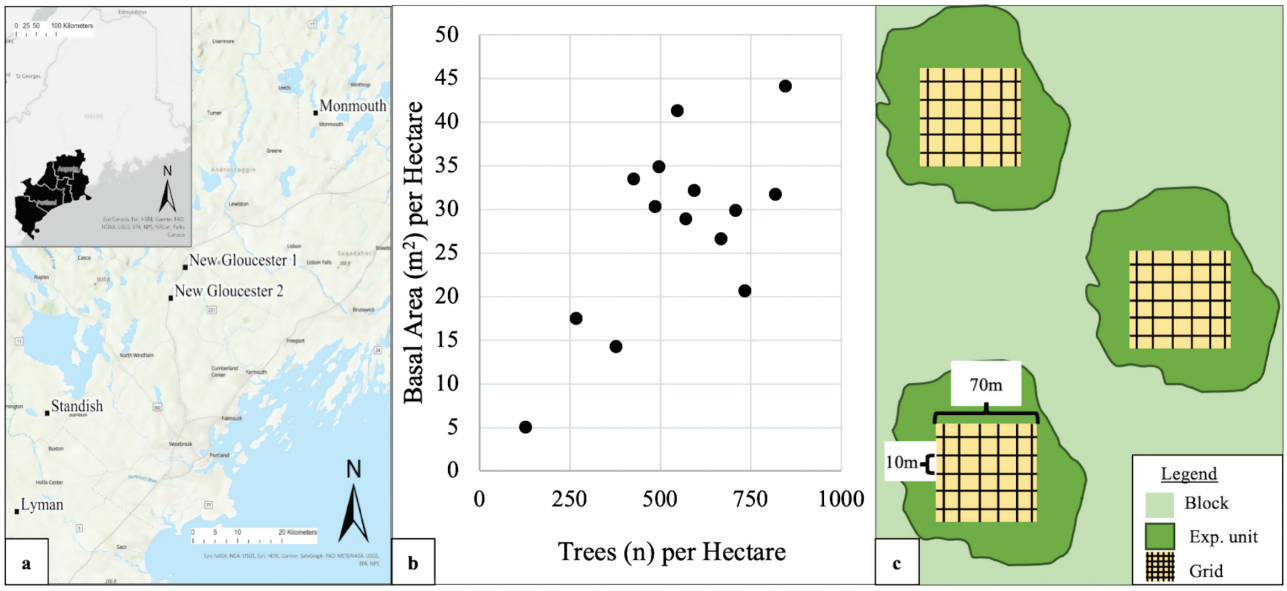

Fig. 1. a) Location of blocks throughout southern Maine. b) Number of trees and basal area per hectare for 14 experimental units. c) Example layout of one block, showing the sampling grid within each of the three 1-ha experimental units within each block.

(Leak et al. 2014, Maine DACF 2021). Common species included northern red oak (*Quercus rubra*), American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), red maple (*A. rubrum*), eastern white pine (*Pinus strobus*), and eastern hemlock (*Tsuga canadensis*). Each block contained 3 experimental units with different timber harvesting histories, though no harvest occurred more recently than 5 yr prior and none were clear cuts, only partial timber harvests (i.e., the predominant approach in Maine where some, but not all, overstory trees are removed). These varied harvesting histories provided a range of current forest stand conditions, encompassing a gradient of low numbers of trees and/or basal area per hectare to high numbers of trees and/or basal area per hectare. To ensure these diverse stand conditions were represented in the study, during the site selection process we calculated the number of trees and the basal area per hectare for each site (Fig. 1b, Table 1) by conducting overstory measurements (Husch et al. 2003) at 5 randomly selected points in each experimental unit. In each experimental unit, we established a 70 m × 70 m grid spaced at 10 m intervals in the middle of the hectare to avoid edge effects (Fig. 1c). We set up these grids to guide data collection and to allow us to measure the distance we traveled on each visit when collecting ticks. To test our hypotheses, in each experimental unit we collected data on tick densities, forest stand structure, microhabitat features, and daily deer activity.

Tick Densities and Infection Prevalence

To test the hypothesis that the number of trees and basal area per hectare relate to tick densities, we performed drag sampling (Falco and Fish 1992, Daniels et al. 2000), wherein a 1 m^2 corduroy cloth is dragged across leaf litter and vegetation. As drag sampling only collects host-seeking (i.e., questing) ticks, hereafter mention of tick densities refers specifically to questing tick densities. We visited each experimental unit once every 2 wk throughout the study period from 24 June to 21 August 2019, and 23 June to 27 August 2020. We dragged between 10:00 and 16:00 hours, only on sunny days with no rainfall (Ostfeld et al. 1995). During each sampling event, we dragged between 2 and 6 randomly selected 70 m transects (i.e., for a total of 140–420 m^2). We avoided dragging the same transect lines in consecutive 2-wk intervals to ensure even coverage of the grid

Table 1. The number of trees and basal area per hectare of trees 8.9 cm or greater in diameter for each experimental unit

Block	Site name	Trees (*n*) per hectare	**Basal area (m^2) per hectare**
1	New Gloucester A—1	669.2	26.6
1	New Gloucester A—2	378.6	14.2
1	New Gloucester A—3	847.0	44.1
2	New Gloucester B—1	497.0	34.9
2	New Gloucester B—2	733.3	20.7
2	New Gloucester B—3	708.3	29.9
3	Lyman—1	546.9	41.3
3	Lyman—2	267.5	17.5
3	Lyman—3	818.7	31.7
4	Monmouth—1	595.2	32.2
4	Monmouth—2	485.5	30.3
5	Standish—1	570.5	28.9
5	Standish—2	129.0	5.0
5	Standish—3	426.5	33.5

over the course of the field season. We dragged a total of 30,590 m^2 over the course of the study (15,750 m^2 in 2019 and 14,840 m^2 in 2020). Ticks of all species and life stages were removed from the cloth using forceps and preserved in 70% ethanol in a freezer. To calculate tick densities, we standardized the total number of ticks collected per experimental unit per 100 m^2. We excluded larvae and adults in our analyses as they were not the life stages of interest and we did not collect during their peak activity. We also excluded other tick species besides *I. scapularis* from our analyses because they were not the species of interest. Ticks were sexed and identified to species using taxonomic keys (Keirans and Litwak 1989, Levin et al. 1997) and a Leica EZ4W stereo microscope (Leica Microsystems Inc., Chicago, IL).

To test the hypothesis that the number of trees and basal area per hectare relate to nymphal infection prevalence, a subset of nymphs was tested for pathogens, including *B. burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia microti*, and *Borrelia miyamotoi*. As the Centers for Disease Control and Prevention

(CDC) guidelines suggest testing a minimum of 25 nymphs to estimate pathogen infection prevalence (CDC 2020), 30 nymphs were randomly selected from each experimental unit for each year. If fewer than 25 nymphs were available, all nymphs were tested but the data were excluded from statistical analysis. If more than 30 nymphs were available, then 30 were randomly selected for testing by assigning arbitrary numbers to each vial containing ticks and then using a random number generator to select vials until we reached a total of 30 nymphs. Nymphs were sent to the University of Maine Cooperative Extension Diagnostic and Research Laboratory (Orono, ME). There, tick DNA was extracted and then pathogens were detected using a multiplex quantitative polymerase chain reaction assay following the procedures described in Rounsville et al. (2021).

Forest Measurements

To test the hypotheses that the number of trees and basal area per hectare relate to ticks through changes to overstory, midstory, and understory features, and forest floor microhabitat features, we measured variables commonly used in forestry (Table 2). Measurements were conducted in each experimental unit as follows. In the overstory, we measured tree species composition of trees that were 8.9 cm in diameter at breast height (i.e., dbh; 1.3 m) and larger (Maine Bureau of Forestry 2008) and canopy closure (Jennings et al. 1999) at 5 randomly selected sampling points using variable-radius sampling plots. We determined canopy closure using a spherical densiometer as described by Jennings et al. (1999). In the midstory, we conducted sapling counts at these same sampling points using 2.5 m fixed-radius sampling plots (Ministry of Forests, Lands and Natural Resource Operations 2016). We counted the species and number of saplings belonging to different class sizes using a "go/no-go" gauge: a custom-built tool that sorts saplings into 1 of 3 size classes (i.e., size class 1: 1.3–3.6 cm, size class 2: 3.7–6.1 cm, or size class 3: 6.2–8.6 cm). Understory measurements were conducted within a 1 m^2 quadrat deployed at these same sampling points and another 5 randomly selected sampling points (for a total of 10 sampling points per experimental unit) due to the smaller spatial scale of these measurements. Within each quadrat, we visually estimated percent cover to the nearest 5% provided by understory vegetation at 2 height ranges (0–0.5 m and 0.6–1.4 m), and recorded understory vegetation type (e.g., herbaceous, shrub, and grass), including noting any invasive plant presence. Finally, on the forest floor at the same 10 sampling points, we measured leaf litter depth and visually estimated the percent of the ground covered by leaf litter and leaf litter composition (i.e., mostly deciduous, coniferous, or mixed).

Microclimate

To test the hypothesis that the number of trees and basal area per hectare are associated with the microclimate of off-host tick habitat within the leaf litter, we deployed 1 iButton data logger (catalog #DS1922L-F5, Maxim Integrated, San Jose, CA) within the leaf litter in the center of each experimental unit to record temperature (°C) and percent relative humidity once per hour from 24 June to 21 August 2019 and 23 June 23 to 27 August 2020. In 2020, we placed a second set of iButtons at 40 cm above the leaf litter at the typical questing height for *I. scapularis* (Tietjen et al. 2020), allowing a comparison of temperature and humidity between the questing habitat and leaf litter habitat. We placed each iButton in a plastic mesh pocket affixed to a field flag embedded in the ground to protect the iButtons from wildlife and loss while remaining exposed to environmental conditions (e.g., sunlight, moisture).

Table 2. Forest structure variable means (±SE) for each site

			Sapling count			Understory vegetation cover		Leaf litter	
Block	Site name	Canopy closure (%)	Diameter size class: 1.3–3.6 cm (*n*)	Diameter size class: 3.8–6.1 cm (*n*)	Diameter size class: 6.4–8.6 cm (n)	Height range: 0–0.5 m (%)	Height range: 0.5–1.4 m (%)	Depth (cm)	Cover (%)
1	New Gloucester A—1	87 ± 3.5	4.2 ± 0.6	0.4 ± 0.0	0.0 ± 0.0	30 ± 4.3	30 ± 8.5	7.0 ± 0.0	96 ± 2.6
1	New Gloucester A—2	84 ± 9.4	5.6 ± 0.7	0.6 ± 0.2	0.2 ± 0.2	45 ± 7.1	17 ± 6.0	7.2 ± 0.4	100 ± 0.67
1	New Gloucester A—3	88 ± 2.8	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	21 ± 4.9	13 ± 9.0	10.1 ± 0.5	90 ± 3.3
2	New Gloucester B—1	89 ± 3.8	1.8 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	56 ± 7.5	34 ± 7.8	10.1 ± 0.6	96 ± 2.2
2	New Gloucester B—2	84 ± 2.2	0.6 ± 0.2	0.8 ± 0.4	0.4 ± 0.2	53 ± 8.1	20 ± 9.0	11.1 ± 0.7	94 ± 2.6
2	New Gloucester B—3	95 ± 2.3	3.2 ± 0.6	0.2 ± 0.2	0.8 ± 0.0	30 ± 4.5	15 ± 8.7	11.1 ± 0.6	97 ± 2.4
3	Lyman—1	91 ± 2.7	2.0 ± 0.9	0.6 ± 0.4	0.0 ± 0.0	1 ± 0.2	8 ± 7.5	11.6 ± 0.7	93 ± 3.0
3	Lyman—2	76 ± 12	8.2 ± 5.0	0.2 ± 0.2	0.2 ± 0.0	56 ± 7.3	17 ± 5.7	10.5 ± 0.6	97 ± 2.5
3	Lyman—3	77 ± 6.3	0.6 ± 0.4	0.4 ± 0.2	0.2 ± 0.2	57 ± 4.8	18 ± 8.7	7.9 ± 0.5	84 ± 8.7
4	Monmouth—1	76 ± 7.0	0.8 ± 0.2	0.4 ± 0.2	0.0 ± 0.0	19 ± 4.6	15 ± 6.1	8.9 ± 0.6	88 ± 5.0
4	Monmouth—2	91 ± 0.42	1.8 ± 0.4	0.8 ± 0.3	0.0 ± 0.0	28 ± 11	19 ± 7.8	8.3 ± 0.7	84 ± 10
5	Standish—1	90 ± 3.2	0.4 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	27 ± 7.9	17 ± 8.9	8.6 ± 0.6	94 ± 3.0
5	Standish—2	45 ± 12	10.0 ± 1.9	5.2 ± 1.2	0.6 ± 0.4	75 ± 8.5	39 ± 10.4	9.0 ± 0.7	93 ± 5.0
5	Standish—3	93 ± 1.5	0.4 ± 0.2	0.8 ± 0.3	0.8 ± 0.6	21 ± 5.2	4 ± 2.8	12.7 ± 0.7	94 ± 3.8

Daily Deer Activity

To test the hypothesis that the number of trees and basal area per hectare relate to the daily activity of deer, we deployed motion-activated trail cameras (Browning Strike Force HD Pro X, catalog #SKUBTC-5HDPX, Browning Trail Cameras, Birmingham, AL) in each experimental unit (Hofmeester et al. 2017) from 24 June to 21 August 2019 and 23 June to 27 August 2020. This sampling period coincides with peak nymphal questing activity, providing a preliminary assessment of the potential availability of this host to questing nymphs. We placed 2 trail cameras per experimental unit in 2019 and 4 trail cameras per experimental unit in 2020 (except for 1 experimental unit, which had 3 trail cameras). In 2019, the 2 trail cameras were relocated within the forest stand every 2–3 wk to ensure coverage of most of the experimental unit. In 2020, with the additional trail cameras deployed, it was unnecessary to rotate the cameras to ensure full coverage. We positioned the cameras, unbaited, at 40 cm above the ground, angled parallel to the ground, to capture the movement of passing deer and other wildlife (Hofmeester et al. 2017). Every 2 wk, we downloaded photos from the cameras, searched through the photos for ones containing motion-captured deer, and calculated daily deer activity.

To assess deer activity, we recorded each instance a deer triggered the trail camera and the number of deer present. While it was possible for deer to pass in front of more than 1 trail camera within an experimental unit, we assumed independence between detection instances by only recording detections that occurred 30 min apart or more. If 2 detections occurred within 30 min for the same trail camera (Kelly and Holub 2008, Wang et al. 2015, O'Connor et al. 2017), we did not consider the events independent, unless it was obvious this was a new individual (adult vs. fawn, deer with antlers vs. without, etc.) (O'Connor et al. 2017). To assess daily deer activity (i.e., daily deer occurrence per 24 h), we divided the total instances of deer for the experimental unit by the total number of trap nights for the experimental unit (Rowcliffe et al. 2008, Rovero and Marshall 2009).

Statistical Analyses

Trees and Basal Area per Hectare vs. Nymphs

To test the hypothesis that the number of trees and basal area per hectare relate to *I. scapularis* nymph densities and infection prevalence, we conducted linear mixed-effects analyses (LMERs) using the lme4 package (Bates et al. 2015) in R version 4.0.2 (R Core Team 2020). The number of trees per hectare and basal area per hectare were highly correlated ($r = 0.71$) so only trees per hectare were retained as a predictor variable to avoid issues of multicollinearity. One model included nymph densities as the response variable, treated the number of trees per hectare and the time interval of tick collection as fixed effects, and treated the collection year and block as random effects. Nymph densities were calculated as means per experimental unit, separated out by each collection interval for each study year ($n = 140$). To test for the relationship between the number of trees and basal area per hectare on infection prevalence, the same model above was used instead as a generalized linear mixed-effects model (GLMER) with a binomial distribution and the binary response variable of either infected or uninfected for each tested nymph ($n = 364$). We only included in our analyses data from experimental units where we collected at least 25 nymphs in accordance with CDC guidelines, which gives 80% power to detect a prevalence of 6.2% or higher (CDC 2020). Therefore, we only included year 1 nymphal infection prevalence in our model, except for 2 experimental units (Lyman 1 and Standish 3, Table 5) and so excluded the random effect of collection year from the model. Separate models were run for nymphal infection prevalence for each pathogen (i.e., *B. burgdorferi* s.l., *A. phagocytophilum*, *B. microti*, and *B. miyamotoi*).

Trees and Basal Area per Hectare vs. Forest Structure

To test the hypotheses that the number of trees and basal area per hectare are related to overstory, midstory, understory, and forest floor structure, we conducted multivariate analysis of variance (MANOVA) in R using the package ggfortify (Tang et al. 2016) to analyze the relationship between the number of trees and basal area per hectare on forest structure variables. In the MANOVA model, the predictor variables were the number of trees per hectare and basal area per hectare, and the response variables were forest structure variables (canopy closure, sapling counts for all 3 size classes, understory vegetation cover at 2 height ranges, and leaf litter cover and depth). To visually represent the relationships among the various forest structure variables, we conducted a principal components analysis (PCA) in R using the package ggfortify.

Trees and Basal Area per Hectare vs. Microclimate

To test the hypothesis that the number of trees and basal area per hectare relate to the microclimate of off-host tick habitat, we used MANOVA in R. In the MANOVA model, the predictor variables were the number of trees per hectare and basal area per hectare, the response variables were the 6 microclimate variables of mean, minimum, and maximum for both temperature and humidity, and the time interval of tick collection was treated as a fixed effect. For each experimental unit, we aggregated the microclimate data by each 2-wk tick collection interval in both years. To calculate these variables, for each collection interval for both temperature and humidity we calculated the overall mean of all reported values, the lowest value reached, and the highest value reached. We ran 1 MANOVA for the microclimate variables in the leaf litter and a separate MANOVA for microclimate variables in the questing habitat. To visually represent the relationships among the microclimate variables, we conducted PCAs for both the leaf litter microclimate and the questing habitat microclimate.

Trees and Basal Area per Hectare vs. Deer

To test the hypothesis that the number of trees and basal area per hectare relate to the daily activity of deer, we used GLMER with a Gamma distribution. We aggregated the deer data by taking the means for each experimental unit per each study year ($n = 28$). The model included the number of trees and basal area per hectare as the predictor variables and block and collection year as random effects.

Top Forest Structure Predictors of Nymphs

To elucidate potential forest structure drivers of nymph densities, we further analyzed which of the forest structural variables may be the best predictors of nymph densities with a random forest model using the randomForest package (Liaw and Wiener 2002) in R. Random forest is a machine learning statistical technique that is appropriate for non-linear relationships, handles collinearity between independent variables well, and can predict continuous dependent variables (Breiman 2001). Nymph densities were calculated as means per experimental unit, separated out by each collection interval for each study year ($n = 140$). In this model, the response variable was nymph densities, and the predictor variables were blocked, the time interval of tick collection, and all forest structural attributes listed in Tables 1 and 2, plus forest composition features like dominant

tree species, dominant sapling species, dominant understory vegetation type (shrub, herbaceous, tree, fern, or grass), and the dominant leaf litter type (coniferous, deciduous, or mixed). The presence of invasive plants was excluded as the presence was extremely low or absent across all sites. Variable importance (i.e., a measure of how each variable's partitioning of the data at each decision tree in the random forest best improves the model's predictiveness of nymph densities) and mean squared error (MSE) were used to identify forest variables driving nymph densities. Bootstrapping was used for internal model validation. Three-quarters of the field-collected data were used to train the model, and the remaining quarter was used to test the model. To assess model performance, we compared the training MSE to the testing MSE.

Microclimate vs. Nymphs

To determine whether microclimate relates to nymph densities, we used LMER to analyze the effects of the microclimate variables on nymph density. We ran separate LMERs for the microclimate variables in the leaf litter and the microclimate variables within the questing habitat. Nymph densities were calculated as means per experimental unit, separated out by each collection interval for each study year (n = 140). The leaf litter microclimate LMER included nymph densities as the response variable, all 6 leaf litter microclimate variables (temperature and humidity mean, minimum, and maximum) as predictor variables, time interval of tick collection as a fixed effect, and collection year and block as random effects. The questing microclimate LMER contained the same effects except used the 6 microclimate variables within questing habitat instead and did not include year as a random effect, because data on questing habitat microclimate were collected during only 1 yr of the study.

Deer vs. Nymphs

Finally, to determine whether deer activity relates to nymph densities, we used an LMER using the lme4 package (Bates et al. 2015) in R to analyze the effect of daily deer activity on the density of nymphs. We aggregated the deer and nymph data by taking the means for each experimental unit per each study year (n = 28). The model included block and the collection year as random effects.

Results

Tick Densities and Infection Prevalence

We collected a total of 991 host-seeking *I. scapularis* throughout the 2-yr study (Table 3). In 2019, we collected 713 nymphs and 178 adults from all sites, and in 2020, we collected 64 nymphs and 36 adults. We also collected 319 adult American dog ticks, *Dermacentor variabilis* Say, and 7 adult rabbit ticks, *Haemaphysalis leporispalustris* Packard, in 2019, and 133 adult *D. variabilis* in 2020. These latter species and adult *I. scapularis* were excluded from statistical analyses because they were not the key species or life stage of interest in this study. We found a positive relationship between the number of trees per hectare and nymph densities (β = 1.34, SE = 0.60, t = 2.21, P = 0.03, n = 140) (Table 4, Fig. 2), indicating as the number of trees per hectare increased by 1, the mean nymph density increased by 1.34 nymphs.

Table 3. The total counts of ticks by species and life stage for each experimental unit, all collection intervals aggregated, per study year

			Ixodes scapularis			Dermacentor variabilis	Haemaphysalis leporispalustris
Year	Block	Site name	**Nymph density (n/100 m^2)**	Total nymphs	Total adults	Total adults	Total adults
1	1	New Gloucester A—1	4.57	48	30	13	0
1	1	New Gloucester A—2	4.48	47	16	30	1
1	1	New Gloucester A—3	4.67	49	4	4	0
1	2	New Gloucester B—1	3.05	32	8	34	0
1	2	New Gloucester B—2	6.38	67	10	48	1
1	2	New Gloucester B—3	3.71	39	14	34	0
1	3	Lyman—1	1.05	11	0	9	0
1	3	Lyman—2	3.43	36	20	21	1
1	3	Lyman—3	5.62	59	4	20	1
1	4	Monmouth—1	8.19	86	11	8	0
1	4	Monmouth—2	7.14	75	22	31	0
1	5	Standish—1	6.48	68	4	2	0
1	5	Standish—2	2.29	24	14	22	2
1	5	Standish—3	1.14	12	4	17	1
2	1	New Gloucester A—1	3.81	6	1	1	0
2	1	New Gloucester A—2	4.29	6	2	3	0
2	1	New Gloucester A—3	1.67	3	3	3	0
2	2	New Gloucester B—1	0.71	1	5	13	0
2	2	New Gloucester B—2	5.43	10	1	28	0
2	2	New Gloucester B—3	2.02	5	2	8	0
2	3	Lyman—1	0.36	1	0	6	0
2	3	Lyman—2	2.50	6	4	18	0
2	3	Lyman—3	4.36	12	1	10	0
2	4	Monmouth—1	0.86	3	0	3	0
2	4	Monmouth—2	0.36	1	6	13	0
2	5	Standish—1	1.79	3	0	0	0
2	5	Standish—2	2.43	4	10	22	0
2	5	Standish—3	1.43	5	0	2	0

We detected 4 tick-borne pathogens in field-collected *I. scapularis* nymphs study-wide (Table 5). The highest proportion of nymphs were positive for *B. burgdorferi* s. l. (22.9%), followed by *A. phagocytophilum* (5.8%), *B. microti* (5.3%), and *B. miyamotoi* (0.4%). As *B. miyamotoi* is broadly included in the Borrelia bacteria group, *B. miyamotoi* infected nymphs also showed as positive for *B. burgdorferi*. Our assay determined whether a *B. burgdorferi* sensu lato positive nymph also contained *B. miyamotoi* but could not distinguish whether there was a co-infection with one of the other *Borrelia* bacteria, as no others were included in the testing panel. Three ticks were coinfected with *B. burgdorferi*, *A. phagocytophilum*, and *B. microti*, 14 with *B. burgdorferi* and *A. phagocytophilum*, and 16 with *B. burgdorferi* and *B. microti* (Table 5). From the year 1 collected nymphs (n = 364), there was no significant effect of the number of trees per hectare on nymphal infection prevalence for *B. burgdorferi* (β = 0.001, SE = 0.001, z = 0.70, P = 0.49), *A. phagocytophilum* (β = –0.002, SE = 0.003, z = –0.62, P = 0.54), *B. microti* (β < 0.0001, SE < 0.001, z = 0.01, P = 99), or *B. miyamotoi* (β = –0.004, SE = 0.01, z = –0.33, P = 74).

Table 4. The results of the LMER model examining the effect of number of trees per hectare on nymph densities. Asterisk denotes statistical significance at α = 0.05

Fixed effects					
Variable	Estimate	SE	*df*	*t*-value	*P*-value
Intercept	2.60	2.13	1.29	1.23	0.40
Trees per hectare	1.34	0.60	113.00	2.21	0.03*
Interval	–0.45	0.13	131.50	–3.43	0.001*
Random effects					
Variable	**Variance**		**Standard deviation**		
Location	0.36		0.60		
Year	7.87		2.81		

Forest Measurements

Overall, greater numbers of trees and/or basal area per hectare were associated with greater overstory canopy closure and leaf litter depth, but lower counts of midstory saplings, understory vegetation cover, and amount of ground covered by leaf litter on the forest floor (Table 6, Fig. 3). The PCA suggested relationships between overstory structure (i.e., number of trees and basal area per hectare) and other forest structural variables (Fig. 3). The first principal component (PC1) explained 49.96% of variance in the dataset and PC2 explained 18.25%. The top 4 PCs accounted for 87.19% of variation. PC1 showed the strongest positive associations (factor loading ≥ 0.33) with basal area per hectare, canopy closure, and number of trees per hectare (Table 7). PC2 showed the strongest positive associations (factor loadings ≥ 0.11) with understory vegetation cover at both heights and number of trees per hectare (Table 7).

Microclimate and Daily Deer Activity

Overall, the number of trees and basal area per hectare related to leaf litter microclimate variables (Table 8, Fig. 4a). Greater numbers of trees or basal area per hectare correlated with higher minimum humidity and temperature and lower maximum temperature and average humidity (Fig. 4a). This indicates that leaf litter temperature is less variable with a greater number of trees per hectare. In the leaf litter PCA, PC1 explained 27.03% of variance in the dataset and PC2 explained 20.62%. The top 4 PCs accounted for 77.53% of variation. PC1 showed the strongest positive associations (factor loading ≥ 0.12) with leaf litter minimum humidity, average humidity, and the time interval of tick collection (Table 9). PC2 showed the strongest positive associations (factor loadings ≥ 0.38) with the number of trees per hectare, basal area per hectare, and leaf litter minimum temperature (Table 9). The number of trees and basal area per hectare were also related to questing habitat microclimate variables

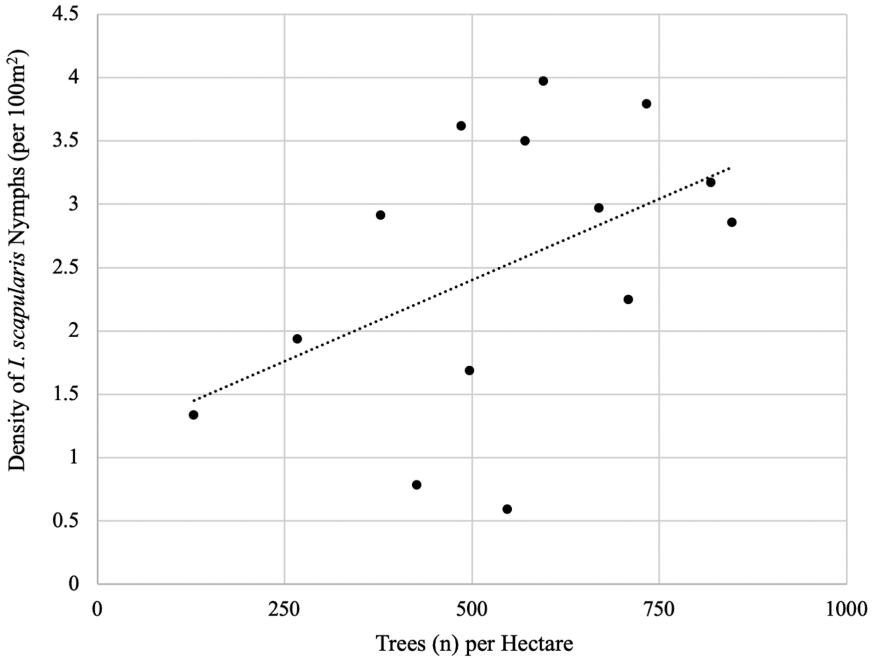

Fig. 2. The density of nymphs per 100 m², combined for both 2019 and 2020, versus the number of trees per hectare.

Table 5. Number of *I. scapularis* nymphs collected (Collected) and tested (Tested) for pathogens per site for 2019 and 2020, and proportion of nymphs infected with *Borrelia burgdorferi* sensu lato (Bb), *Anaplasma phagocytophilum* (Ap), *Babesia microti* (Bmic), *Borrelia miyamotoi* (Bmiy), or multiple pathogens

Year	Block	Site name	Collected	Tested	Bb	Ap	Bmic	Bmiy	Bb/Ap/Bmic	Bp/Ap	Bb/Bmic
1	1	New Gloucester A—1	48	38	0.37	0.11	0.05	0	0	0.01	0.05
1	1	New Gloucester A—2	47	30	0.30	0	0.03	0	0	0	0.03
1	1	New Gloucester A—3	49	30	0.23	0.03	0.03	0	0	0.03	0.03
1	2	New Gloucester B—1	32	30	0.20	0	0.03	0.03	0	0	0.03
1	2	New Gloucester B—2	67	30	0.33	0.07	0	0	0	0.07	0
1	2	New Gloucester B—3	39	30	0.17	0.03	0	0	0	0.03	0
1	3	Lyman—1	11	11	0.18	0	0	0	0	0	0
1	3	Lyman—2	36	30	0.27	0.17	0.10	0	0.03	0.07	0.07
1	3	Lyman—3	59	30	0.27	0.07	0.13	0	0	0	0.13
1	4	Monmouth—1	86	30	0.27	0.03	0.13	0	0.03	0	0.07
1	4	Monmouth—2	75	30	0.23	0	0.07	0	0	0	0.03
1	5	Standish—1	68	30	0.10	0.07	0	0	0	0	0
1	5	Standish—2	26	26	0.15	0.08	0.04	0	0	0.08	0
1	5	Standish—3	12	12	0.33	0.08	0.17	0.08	0	0.08	0
2	1	New Gloucester A—1	6	6	0.33	0.17	0	0	0	0.17	0
2	1	New Gloucester A—2	6	6	0.17	0	0	0	0	0	0
2	1	New Gloucester A—3	3	3	0	0	0	0	0	0	0
2	2	New Gloucester B—1	1	1	0	0	1	0	0	0	0
2	2	New Gloucester B—2	10	10	0.20	0	0	0	0	0	0.10
2	2	New Gloucester B—3	5	5	0.20	0	0	0	0	0	0
2	3	Lyman—1	1	1	0	0	1	0	0	0	0
2	3	Lyman—2	5	5	0.20	0.40	0	0	0.2	0	0
2	3	Lyman—3	12	12	0	0.17	0	0	0	0	0
2	4	Monmouth—1	3	3	0	0	0	0	0	0	0
2	4	Monmouth—2	1	1	0	0	0	0	0	0	0
2	5	Standish—1	3	3	0	0	0	0	0	0	0
2	5	Standish—2	4	4	0.25	0	0.25	0	0	0	0.25
2	5	Standish—3	2	2	0	0	0	0	0	0	0

Table 6. The results of the MANOVA model examining the effect of number of trees and basal area per hectare on forest structure variables. Asterisk denotes statistical significance at $\alpha = 0.05$

Predictor variable	Pillai's trace	Approx. *F*	Num. *df*	Den. *df*	*p*
Trees per hectare	0.94	28.18	20	38	<0.0001*
Basal area	0.98	79.95	20	38	<0.0001*

(Table 8, Fig. 4b). Greater numbers of trees or basal area per hectare correlated with higher average humidity and minimum temperature, and lower average and maximum temperature (Fig. 4b). Again, this indicates that temperature is less variable with greater numbers of trees per hectare. In the questing habitat PCA, PC1 explained 25.35% of variance in the dataset and PC2 explained 21.85%. The top 4 PCs accounted for 77.63% of variation. PC1 showed the strongest positive associations (factor loading ≥ 0.44) with average, maximum, and minimum temperatures (Table 9). PC2 showed the strongest positive associations (factor loadings ≥ 0.41) with the number of trees per hectare, basal area per hectare, and average humidity (Table 9).

Neither number of trees per hectare (β = 0.003, SE = 0.02, t = 0.12, df = 27, P = 0.90) nor basal area per hectare (β = –0.06, SE = 0.04, t = –1.55, df = 27, P = 0.12) were correlated with daily deer activity.

Predictors of Nymph Densities

The random forest model compared the ability of different forest structure variables to predict nymph densities. The strongest predictors of nymph densities were collection interval and understory structure variables, including understory vegetation cover, leaf litter cover, and leaf litter depth (Fig. 5). The model did not overfit as the training MSE (3.39) and testing MSE (3.88) were similar, which was expected as random forests are resistant to overfitting (Breiman 2001).

Only questing microclimate variables were correlated with the density of nymphs; no leaf litter microclimate variables proved significant (Table 10). In the questing habitat, nymph densities positively correlated with average humidity and negatively correlated with minimum humidity.

Deer activity did not have a significant relationship with the density of nymphs (β = –5.80, SE = 6.97, t = –0.83, df = 25.19, P = 0.41).

Discussion

Forest owners and their foresters make management decisions that alter forest structure with consequences for tick populations. This study shows that *I. scapularis* nymph densities vary across stands with different structural conditions. The number of trees per hectare was positively associated with nymph densities. The magnitude of this effect indicates that as the number of trees per hectare increased by 1, the mean nymph density increased by 1.34 nymphs per 100 m^2. Furthermore, findings suggest that effects of the number of trees and basal area per hectare cascade down through the vertical layers of the forest, influencing structural attributes of the overstory, midstory, understory, and forest floor, as well as microclimate conditions of ticks' off-host habitat within the leaf litter and their questing habitat above the forest floor. Specifically, this study's findings suggest that greater numbers of trees or basal area per hectare result in increased

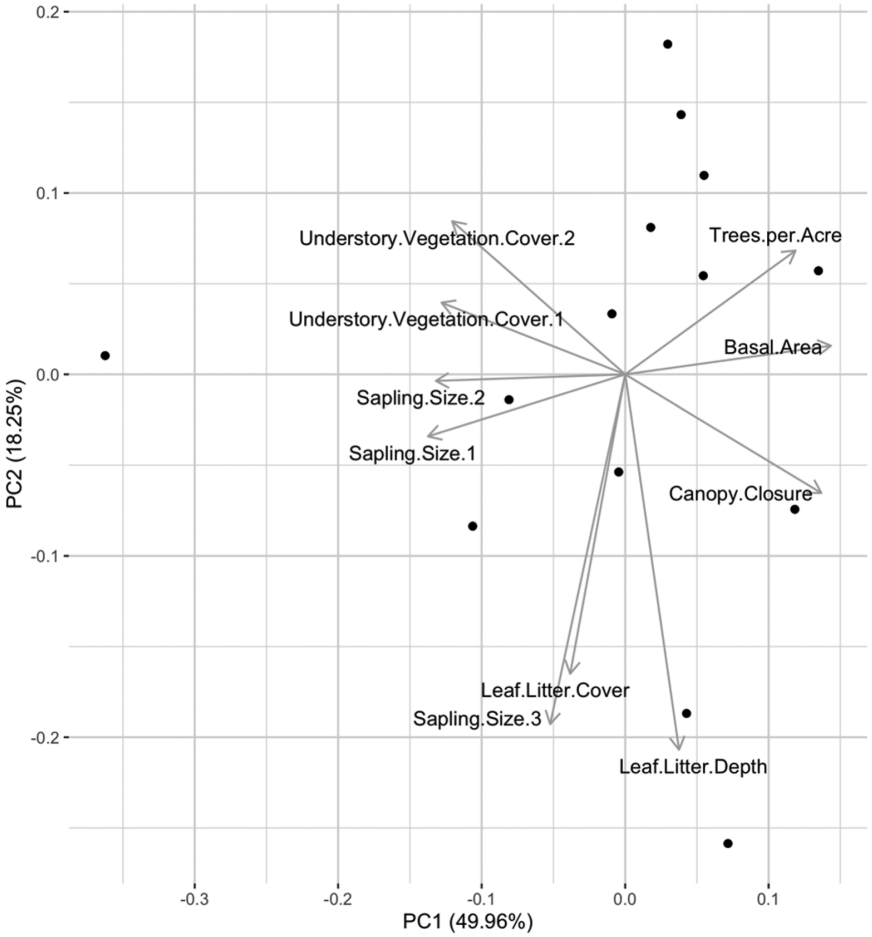

Fig. 3. Plot of PCA showing relationships between all forest structure variables measured.

Table 7. Factor loadings for the top 4 principal components

	Principal component			
Factor	PC1	PC2	PC3	PC4
Basal area	0.40	0.04	−0.17	0.06
Trees per hectare	0.33	0.19	−0.15	−0.51
Canopy closure	0.39	−0.18	0.26	−0.15
Sapling count (1.3–3.6 cm)	−0.39	−0.10	0.26	0.25
Sapling count (3.8–6.1 cm)	−0.37	−0.01	−0.39	0.26
Sapling count (6.4–8.6 cm)	−0.15	−0.54	−0.37	−0.26
Leaf litter depth	0.11	−0.58	−0.36	−0.02
Leaf litter cover	−0.11	−0.46	0.63	−0.20
Understory vegetation cover (0–0.5 m)	−0.36	0.11	−0.02	−0.58
Understory vegetation cover (0.5–1.4 m)	−0.34	0.24	−0.06	−0.38

canopy closure, decreased sapling counts, and understory vegetation cover, which seemed to stabilize the temperature within the leaf litter and questing habitat by reducing temperature peaks and raising temperature minimums, and increased humidity in the leaf litter and questing habitat. A potential pathway linking more trees per acre to higher nymph densities is that greater canopy closure may stabilize the temperature and retain moisture in the questing habitat, increasing the average humidity. As questing habitat average humidity positively correlated to nymph densities, this may create more favorable questing conditions that support tick survival and host encounters, thus facilitating higher questing nymph densities. Additional exploratory analyses suggest that of the forest structure variables evaluated, those that may be driving nymph densities are ones at lower forest layers, specifically the low growing understory vegetation cover and the leaf litter depth and cover along the forest floor.

This research builds upon past studies to investigate a gradient of a number of trees and basal area per hectare resulting from different timber harvesting histories. Furthermore, our findings suggest that harvesting histories that occurred more than 5 yr prior still have consequences for the current stand structure as it relates to nymph densities. The long-term effects of timber harvesting that results in the reduced numbers of overstory trees per hectare represented in this study, may include lower tick densities in those forest stands. This suggests that manipulating overstory density through harvesting may be a useful management tool in mitigating tick densities. This study adds to a growing body of knowledge by further distinguishing between forest structure variables with regard to the strength of their associations with nymph densities, providing a deeper understanding of the potential cascading effects of timber harvesting as a means of manipulating forest structure, microclimate, and resulting nymph densities.

Consistent with past research, we found differences in forest structure (López-Pérez et al. 2021, Brennan et al. 2023), microclimate (Conte et al. 2021, Brennan et al. 2023), and tick densities (Conte et al. 2021, López-Pérez et al. 2021, Brennan et al. 2023) in stands with different timber harvesting histories. Though certain timber harvesting variables like harvest intensity, lapse time since cut,

Table 8. The results of the MANOVA models examining the effect of number of trees and basal area per hectare on microclimate variables in the leaf litter and within questing habitat. Asterisk denotes statistical significance at $\alpha = 0.05$

Leaf litter microclimate						Questing habitat microclimate					
Predictor variable	Pillai's trace	Approx. *F*	Num. *df*	Den. *df*	*P*-value	Predictor variable	Pillai's trace	Approx. *F*	Num. *df*	Den. *df*	*P*-value
Trees per hectare	0.09	2.19	6	131	0.02*	Trees per hectare	0.23	2.55	6	51	0.03*
Basal area	0.10	2.45	6	131	0.03*	Basal area	0.24	2.66	6	51	0.03*
Interval	0.09	2.04	6	131	0.07	Interval	0.54	11.5	6	51	<0.001*

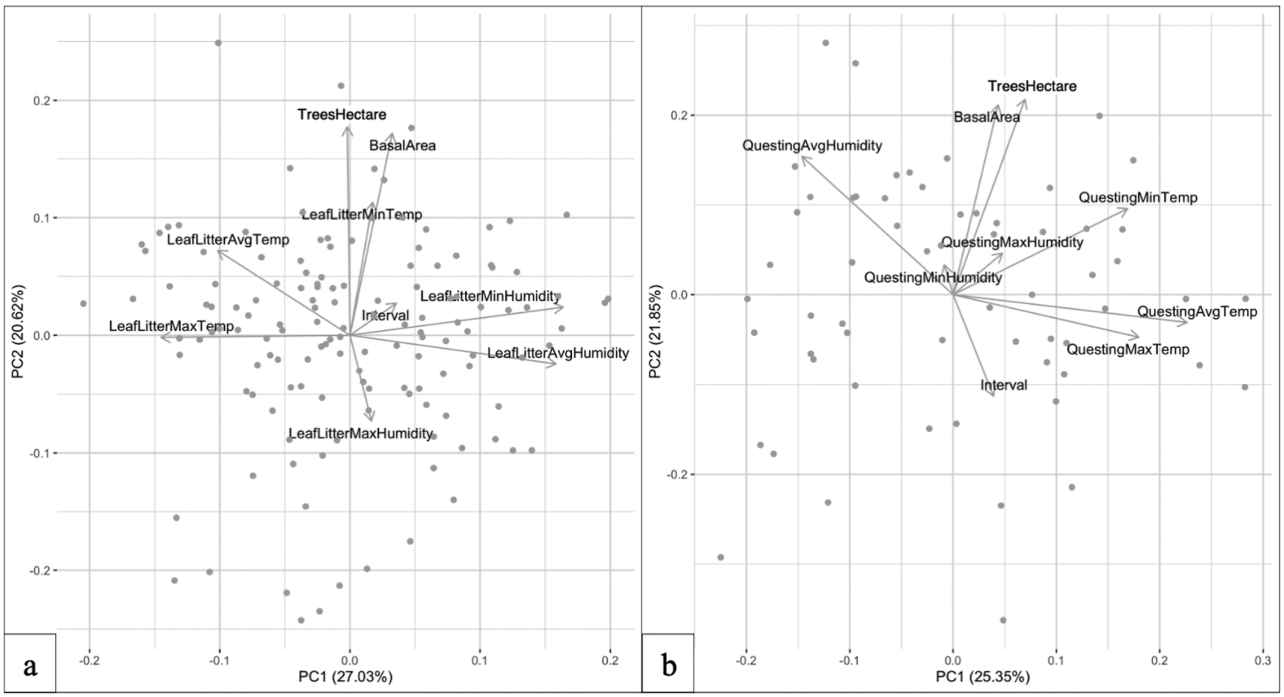

Fig. 4. PCA plots showing relationships between number of trees per hectare, basal area per hectare, and all microclimate variables in a) the leaf litter and b) questing habitat.

or season of operation were not explicitly included in our analyses, forest structure is invariably related to timber harvesting in managed stands. We thus infer a relationship between harvesting, structure, and tick populations that is consistent with previous studies, which showed reduced tick densities in partially harvested stands (Conte et al. 2021, López-Pérez et al. 2021, Brennan et al. 2023) and increased tick densities with increasing canopy closure (Ginsberg et al. 2020). Those studies examined qualitative harvest conditions, examining forest stands 5 yr post-harvest versus those not harvested in the past 20 yr (Conte et al. 2021), 3 yr post-harvest versus not harvested in the past 24 yr (López-Pérez et al. 2021), or 1–3 yr post-harvest versus unharvested (Brennan et al. 2023). Comparatively, our study focused on the quantitative variables (i.e., number of trees per hectare, basal area per hectare) that resulted from different histories of timber harvesting. López-Pérez et al. (2021) also measured canopy closure, and Brennan et al. (2023) measured canopy cover, percent of oak trees per sampling plot, basal area per hectare, trees per hectare, percent understory cover, understory height, and average leaf litter depth. Our study builds upon this past research by measuring additional forest structure variables and performing further analyses linking these variables to tick abundance. Ginsberg et al. (2020) also analyzed the relationships between specific forest structure variables like canopy cover, tree density, shrub density, ground vegetation cover, and litter cover, and tick abundance, though that study was not performed in the context of timber harvesting. Specifically, Ginsberg et al. (2020) found canopy cover to be the best predictor of questing larval tick numbers, but differences in canopy cover were not due to any reported timber harvest. The past timber harvesting studies show the importance of canopy closure in correlations with tick abundance yet did not detect significant relationships between forest structure variables at lower forest layers, like understory vegetation or leaf litter cover, and tick abundance. Our study builds upon this body of work by examining a continuous gradient of numbers of trees and basal area per hectare rather than binary harvested versus unharvested stands, and we also identify drivers of nymph densities in the lower forest layers (i.e., understory vegetation cover and leaf litter depth and cover).

The microhabitats ticks occupy off-host influence tick-host interactions and tick survival (Randolph and Storey 1999, Berger et al. 2014, Burtis et al. 2019). The ideal molting temperature for *I. scapularis* nymphs is 24 °C (with a range of 16–30 °C), and these microclimate conditions can enhance or reduce tick survival (Ogden et al. 2004). The wetter conditions found in this study in sites with more trees per hectare are consistent with past findings of increased humidity in unthinned forests (Brennan et al. 2023). Humidity is known to affect *I. scapularis* nymphs and has been included in the best model predicting nymph densities (McBride et al. 2023). At relative humidity rates below 82%, *I. scapularis* survival

Table 9. Factor loadings for the top 4 principal components for the leaf litter and questing habitat PCAs

Leaf litter microclimate				
	Principal component			
Factor	PC1	PC2	PC3	PC4
Interval	0.12	0.09	−0.01	0.68
Basal area	0.11	0.59	−0.27	−0.22
Trees per hectare	−0.01	0.60	−0.17	−0.28
Average humidity	0.54	−0.08	0.17	−0.26
Maximum humidity	0.06	−0.25	0.43	−0.47
Minimum humidity	0.56	0.08	0.18	0.01
Average temperature	−0.35	0.25	0.54	0.13
Maximum temperature	−0.49	−0.01	0.07	−0.28
Minimum temperature	0.06	0.38	0.60	0.15
Questing habitat microclimate				
	Principal component			
Factor	PC1	PC2	PC3	PC4
Interval	0.10	−0.30	0.61	−0.24
Basal area	0.11	0.56	0.38	0.15
Trees per hectare	0.18	0.57	0.31	0.15
Average humidity	−0.39	0.41	−0.23	−0.29
Maximum humidity	0.13	0.12	−0.40	−0.21
Minimum humidity	−0.02	0.09	0.19	−0.86
Average temperature	0.60	−0.08	−0.17	−0.10
Maximum temperature	0.47	−0.12	0.11	−0.01
Minimum temperature	0.44	0.25	−0.33	−0.13

and host-seeking activity drop significantly, especially when exposed to dry air for more than 4 h (Rodgers et al. 2007, Berger et al. 2014). Low humidity promotes desiccation and decreases tick survival, reducing overall tick densities. Together, the number of trees and basal area per hectare affect canopy closure, which determines the amount of sunlight that reaches the forest floor and vegetation below the canopy, potentially affecting vegetation cover and leaf litter and questing habitat temperature and humidity. Greater numbers of trees per hectare correlated to stabilized microclimate temperatures in off-host habitats through decreases in maximums but increases in minimums. These results are consistent with past findings that reduced temperature variance in winter conditions supports tick survival (Schappach 2022). The correlation between the numbers of trees per hectare and microclimate suggests that harvesting the appropriate number of trees may disrupt microclimate conditions, destabilizing temperature and reducing humidity, resulting in a less favorable off-host tick habitat. However, this study is limited in its application of iButtons as only one was deployed per experimental unit and may not represent the entire hectare, though we feel these exploratory results begin to investigate potential microclimate mechanisms.

In addition to microclimate, the wildlife community also influences tick densities, survival, and ability to molt to the next life stage. Although not investigated as part of this study, forest management practices may alter host habitat quality, consequently altering host (e.g., small mammals) abundance (Carey and Constance 2001, Chambers 2002, Bogdziewicz and Zwolak 2014), which directly affects tick densities, specifically increasing tick densities with increased host abundance (Ostfeld et al. 2018, Tkadlec et al. 2019, Krawczyk et al. 2020). Past research has shown how forest management practices determine forest structure and composition, altering host habitat with consequences for small mammal abundance. For example, pinyon mouse (*Peromyscus truei* Shufeldt) abundance increased with greater basal area of certain tree species (Chambers 2002), small mammal total abundance was reduced in areas of simplified habitat structure and vegetation community composition following decades of clearcutting and extensive management (Carey and Constance 2001), and, in Europe, small mammal abundance increased following clearcutting (Bogdziewicz and Zwolak 2014). A limitation of our study is the lack of information on key small mammal hosts, particularly as a possible mechanism explaining the observed positive correlation between the number of trees per hectare and tick densities. This correlation could be explained by changes to small mammal host populations, where undisturbed habitat with more trees per hectare supports higher host populations, thus providing greater access to blood meals and, consequently, higher tick densities. Additionally, more trees per acre may increase seed production, further supporting higher small mammal abundance (Ostfeld et al. 2001). Other investigators found that harvested areas with reduced tick densities also had lower small mammal captures per night, though estimated population sizes did not differ between harvested versus unharvested stands (Conte et al. 2021). López-Pérez et al. (2021) found harvesting to decrease small mammal captures in the short-term, within 2 yr post-harvest, but harvested versus unharvested plots became comparable by 3 yr post-harvest. Interannual variation in small mammal population sizes may explain the variation in tick collection numbers between the first and second study years. High rodent abundance has been shown to negatively correlate with nymph densities in the current year but positively correlate with nymph densities the following year (Ostfeld et al. 2018). As rodent population sizes oscillate, and fluctuations in Maine's white-footed mouse populations are greater compared to others in the eastern and midwestern United States (Elias et al. 2004), the interannual variation in tick collection may follow these fluctuating rodent populations. Overall, the long-term effects of timber harvesting on small mammals as they relate to tick populations remain underexplored. The potential role of small mammals as the mechanism connecting timber harvesting to tick densities—through structural modifications that affect their habitat—requires further data collection, including small mammal population size estimates, host activity, and mean individual tick burden.

Forest structural attributes manipulated by management practices are known to affect the habitat of other hosts, like white-tailed deer, as well (Tierson et al. 1985, DeGraaf and Yamasaki 2001). Deer are mostly parasitized by adult *I. scapularis* (Wilson et al. 1985), while the white-footed mouse, deer mouse (*Peromyscus maniculatus* Wagner), and eastern chipmunk (*Tamias striatus* Linnaeus) are key hosts for *I. scapularis* nymphs (Main et al. 1982). While deer are not competent reservoir hosts for pathogens transmitted by *I. scapularis* (Telford et al. 1988), they are important reproductive hosts for ticks and support the adult and nymph life stage (Spielman et al. 1985, Rand et al. 2003). This study did not find associations among deer, ticks, and the number of trees and basal area per hectare, but our experimental units were only 1 ha in size and therefore only a small portion of a typical home range for deer (Tierson et al. 1985). Also, we may have sampled deer activity over too short a time period to detect a relationship between deer and ticks, as a 2-yr lagged correlation is suspected between deer and nymph densities (Ostfeld et al. 2001). Conte et al. (2021) found no significant correlation between harvesting and deer activity, although that study was also conducted on a small spatial scale with harvested areas of less than half a hectare. Timber harvesting at greater spatial scales (e.g., in areas greater than 100 km^2) has been shown to increase winter deer movement

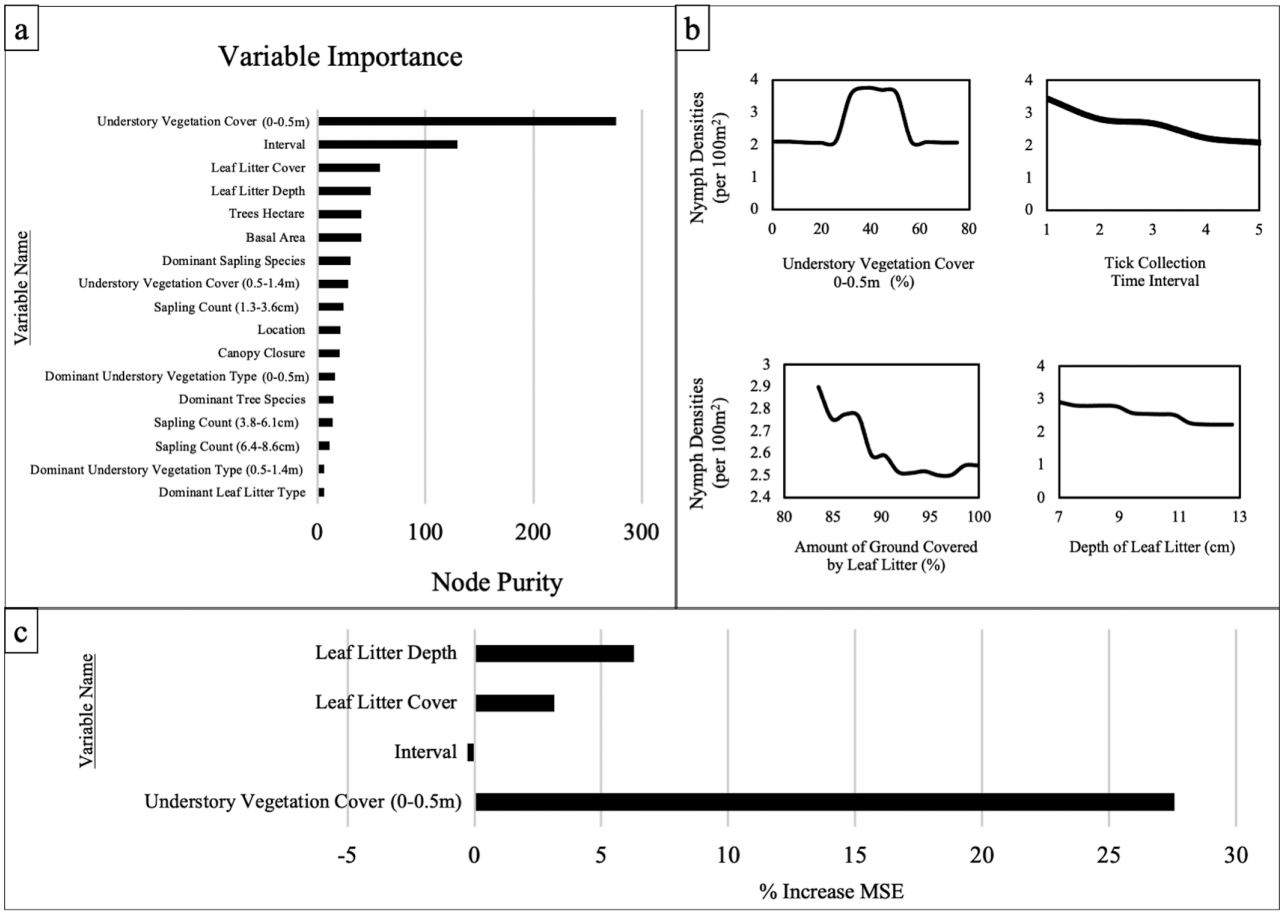

Fig. 5. Results of the random forest model showing a) variable importance plot (variable importance is expressed in terms of percent increase in MSE and percent increase in node purity of each forest structure predictor variable), b) response curves of nymph density for the top 4 predictor variables (first understory vegetation cover followed by tick collection interval, leaf litter cover, and leaf litter depth), and c) the MSE plots for the top 4 forest structure predictor variables.

Table 10. The results of the LMERs examining the correlation between microclimate variables in the leaf litter and questing habitat, and nymph densities. Included are the coefficient estimates (Estimate) and standard errors (SE). Asterisk denotes statistical significance at $\alpha = 0.05$

Habitat	Predictor variable	Estimate	SE	*t*-value	*P*-value
Leaf litter	Intercept	18.35	54.74	−0.05	0.74
	Average humidity	0.001	0.09	1.87	0.99
	Maximum humidity	−0.22	0.58	−0.45	0.71
	Minimum humidity	−0.002	0.02	−2.36	0.94
	Average temperature	0.34	0.25	2.45	0.18
	Maximum temperature	0.04	0.05	−0.21	0.44
	Minimum temperature	−0.09	0.13	0.45	0.49
	Interval	−0.41	0.14	−3.98	<0.01*
Questing habitat	Intercept	−1.22	88.88	−1.43	0.99
	Average humidity	0.08	0.03	0.64	0.01*
	Maximum humidity	−0.09	0.90	1.43	0.92
	Minimum humidity	−0.01	0.01	−1.36	0.04*
	Average temperature	0.11	0.09	−0.24	0.27
	Maximum temperature	0.04	0.04	−1.33	0.29
	Minimum temperature	0.03	0.05	2.96	0.59
	Interval	0.11	0.07	−0.91	0.14

towards, and occupation of, logged home ranges due to increased browsing habitat from felled treetops and creation of trails from logging equipment (Tierson et al. 1985). Similarly, Campbell et al. (2004) speculated that during timber harvesting deer were attracted to harvested stands (>13 ha) to forage on felled tree browse, though timber harvesting did not alter deer movement and home ranges immediately and up to 3 months post-harvest. In Québec, Canada, deer use of a 43-ha logged area was 5 times greater than the average

use of the surrounding deer wintering area (St-Louis et al. 2000). Our study only assessed deer activity at much smaller spatial scales during summer months and not winter. We may have missed modified winter and/or large-scale activity indirectly related to timber harvest through differences in forest structure that are consequential for tick populations, especially adults. Furthermore, our results are specific to questing nymph densities only and cannot be generalized to overall densities, as we only collected nymphs during their peak questing period. Additional studies investigating timber harvesting explicitly, and sampling across seasons and spatial scales on large hosts like deer, smaller hosts like small mammals, and tick populations may elucidate cascading effects across these differing host and vector home ranges.

Our study is additionally spatially limited in its assessment of consequences for forest stands that are adjacent to our experimental units. While we quantified the forest structure and tick densities in our experimental units, we did not examine the conditions of adjacent stands. Potentially, changes within the study stand could impact neighboring stands. Specifically, reduced overstory densities such as those resulting from harvesting may reduce tick densities in the observed stand while allowing for increased densities in the adjacent stands. These effects at the landscape scale on tick populations are unknown. Further investigations that include conditions of neighboring forest stands are necessary to determine any spillover effects into the surrounding landscape from local changes in forest structure.

The development of tools to control tick populations has emerged as a critical research focus (Bernard et al. 2020). Determining forest structural attributes at the property scale that are associated with lower tick densities could inform forest management in creating such tools. The results of this study could inform and be incorporated into management plans that use timber harvesting as a tool in attempts to control tick populations. The most direct way to manipulate forest structure is through timber harvesting. Whether or not one intentionally uses timber harvesting explicitly as a tool for tick control, it can complement landowners' other aims for their forests including realizing income, promoting regeneration, improving hunting and/or recreation, minimizing insect and disease damage, and generating firewood for personal use (Ernst and Knapp 1985, Conway et al. 2003, Butler 2008, Silver et al. 2015). From this study, landowners can understand the consequences of different timber harvesting plans that result in varied forest structural attributes and gain a better understanding of how these altered attributes may impact tick populations on their property. Additionally, one of the novel aspects of this study is that it was conducted using the same forestry measurements foresters use, facilitating more widespread consideration of these results when constructing management plans. As forestry workers are more likely (compared to indoor workers) to find embedded ticks on themselves and to have previously been diagnosed with a tick-borne disease (Roome et al. 2022), they are a key demographic who could benefit from understanding how forest structural attributes that can be manipulated by timber harvesting may change tick densities in the forests they work within.

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