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Belowground herbivory in red pine stands initiates a cascade that increases abundance of Lyme disease vectors

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ABSTRACT

There is increasing recognition that infectious disease patterns are often driven by complex underlying ecological processes. In red pine plantations in the Great Lakes region of North America, feeding by rhizophagous insects triggers a cascade that ultimately results in higher densities of blacklegged ticks, *Ixodes scapularis*, which are the primary vector of the Lyme disease pathogen *Borrelia burgdorferi*. We sampled 31 plantations in Wisconsin, USA, that were diseased or asymptomatic for a previously described tree mortality syndrome that originates with root infestation. Understory vegetation was greater in diseased stands, as were the proportion of samples containing ticks and the number of ticks per sample. Infection rates with *B. burgdorferi* were consistent. Tick densities were identical between declining and healthy portions of symptomatic stands, suggesting stand-level factors are responsible, consistent with mammal movement. These results suggest that forest management practices that affect the dynamics of belowground food webs may have implications for human health.

1. Introduction

Complex ecological processes can yield outcomes that extend beyond the perceived boundaries of the system. For example, deforestation, invasive species, and suburban landscape design can increase the abundance of vectors of human disease pathogens (Frank et al., 1998; Vittor et al., 2009; Swei et al., 2011; Lehmer et al., 2012), bark beetle outbreaks released by warm temperatures can threaten grizzly bear populations by reducing their overwintering food source of whitebark pine cones (Koteen, 2002; Logan et al., 2010), and insect defoliation can alter stream hydrology (Townsend et al., 2004). Unfortunately, our ability to predict such far-reaching outcomes is limited, and their recognition is often *post hoc*. However, there are several commonalities among these examples, including interactions that operate across multiple spatiotemporal scales, thresholds that normally constrain the system within a stable range, and intermittent external drivers that release endogenous positive feedbacks after a critical threshold is exceeded, and thereby alter emergent properties of the system (Peters et al., 2004; Raffa et al., 2008).

Our study focused on a form of habitat conversion – growing conifers in extensive plantations – which is prevalent worldwide. Plantations provide substantial benefits, such as facilitating rapid tree growth, simplifying operational activities, alleviating demands on natural ecosystems, providing employment and economic benefits to rural communities, serving as islands of improved biodiversity in agricultural landscapes, and contributing to carbon sequestration (Gerrand et al., 2003). However, plantations are commonly even-aged monocultures of relatively narrow genetic stock, and are often planted in locations selected for economic rather than ecological factors, both of which can increase susceptibility to damage by insects and pathogens. In contrast, natural forests are more heterogeneous and tend to harbor more numerous and diverse predator communities, which confronts herbivores with difficult challenges in locating and colonizing suitable host trees (Jactel et al., 2002; Jakel and Roth, 2004).

In the Great Lakes region of North America, red pine (*Pinus resinosa* Aiton) is widely grown because of its rapid growth, high uniformity, and ability to grow on marginal soils. Many mature plantations in Wisconsin, USA, are experiencing a syndrome known as 'Red Pine Pocket Decline', which is initiated by native

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root-feeding beetles and their fungal symbionts (Klepzig et al., 1991; Erbilgin and Raffa, 2003; Aukema et al., 2010). Adult *Hylobius radicis* Buchanan, *Hylastes porculus* Erichson, and *Dendroctonus valens* LeConte oviposit in the roots, soil, or basal stem. The larvae partition the resource, developing in the root collar, lateral roots, and basal stem/root collar, respectively. These beetles vector moderately phytopathogenic *Leptographium* spp. fungi into roots (Klepzig et al., 1995). Infection by this insect–fungal complex does not typically kill mature red pine trees, but the resulting stress impairs tree defenses against lethal stem-colonizing bark beetle–fungal complexes (Klepzig et al., 1996; Zhu et al., 2008). *Leptographium* readily grow through a lattice-like system of root grafts below red pine plantations, preceding noticeable above-ground symptoms by 6–8 m (Erbilgin and Raffa, 2003). The resulting gaps expand relatively uniformly and indefinitely, at the rate of several rows of trees per year. As gaps expand, they are colonized by shrubs, forbs, and early-successional woody angiosperms (Aukema et al., 2010) (Fig. 1A).

While the importance of belowground interactions on aboveground processes is becoming increasingly appreciated (Masters et al., 1993; van der Putten et al., 2009), there is a paucity of data regarding their influence on human disease dynamics (Manangan et al., 2007). We used an established ecological research system to examine the intersection of belowground herbivory, plant pathology, aboveground forest ecology, and human epidemiology. Lyme disease is an inflammatory neurological and rheumatoid condition caused by the bacterial pathogen *Borrelia burgdorferi*, which is vectored by the blacklegged tick, *Ixodes scapularis* Say. This tick has a 2-year life cycle (Yuval and Spielman, 1990) and wide host range (Keirans et al., 1996). In the southern area of the Great Lakes region, abundance of larvae peaks in June and August, nymphs peak in June, and adults peak in October. Populations and distributions of *I. scapularis* are increasing across the Midwest, which is the second largest epicenter of Lyme disease incidence in North America (Diuk-Wasser et al., 2010). Reported cases in Wisconsin have risen 6-fold in the last 20 years (Diep Hoang Johnson, WI Dept. of Health Services, pers. comm.), and areas considered at risk are expanding (Estrada-Peña, 2002; Caporale et al., 2005; Diuk-Wasser et al., 2006). Disease incidences of other pathogens vectored by *I. scapularis*, including *Anaplasma phagocytophilum*, *Babesia microti*, and Powassan virus, are also increasing rapidly in the Great Lakes region of North America. Understanding the impact of environmental precursors to tick-borne disease incidence can provide useful insights about emerging and future health risks.

We sampled ticks across red pine sites in central and southern Wisconsin, and report their spatiotemporal variation during two consecutive years. We tested whether habitat changes caused by interactions between below- and aboveground insect herbivores and their symbionts affected the abundance, distribution, and infection rates of *I. scapularis*. We hypothesized that *I. scapularis* population densities would be higher in diseased than asymptomatic stands, owing to the increases in vegetation types conducive to *I. scapularis* and their mammalian hosts. We also hypothesized that *I. scapularis* population densities would be higher in diseased than nonsymptomatic portions of diseased stands, which were located outside the edge of tree mortality. Finally, we hypothesized that *B. burgdorferi* and *A. phagocytophilum* nymphal infection rates would not differ between diseased and asymptomatic stands.

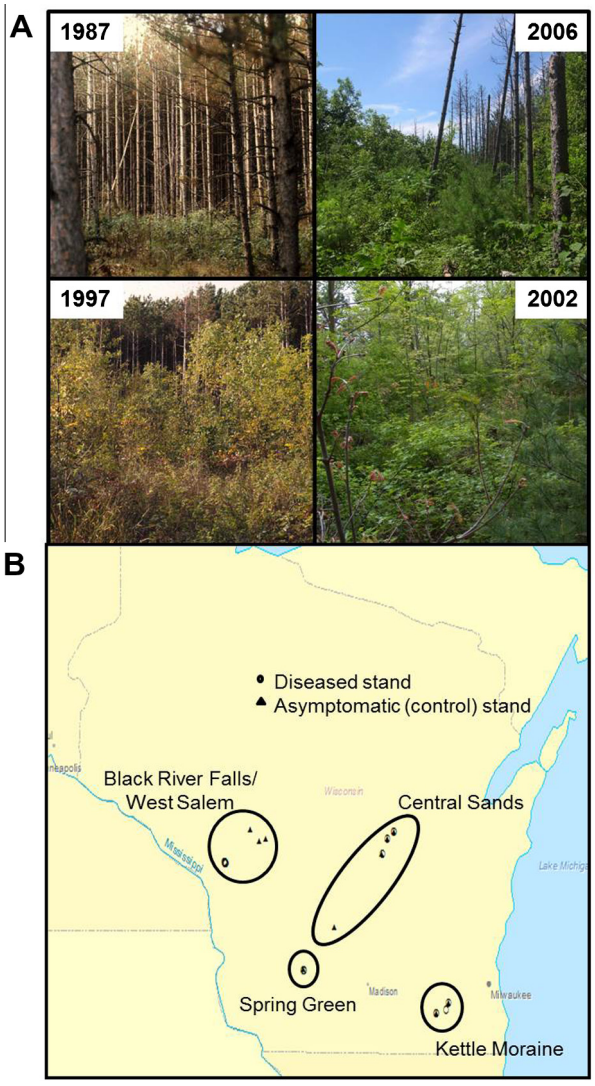

Fig. 1. (A) Gap formation over 20 years caused by interactions among root herbivores, root pathogens, and bark beetles responding to resulting decline in tree defenses. (B) Location of Wisconsin study sites sampled for ticks in 2008 and 2009. Sites were grouped by soil type.

2. Materials and methods

2.1. Study locations and descriptions

Thirty-one mature *P. resinosa* plantations (Table 1), previously studied for their condition and abundance of insects and pathogens causing Red Pine Pocket Decline (Klepzig et al., 1991; Erbilgin and Raffa, 2003; Aukema et al., 2010), were sampled along a transect from west-central to southeastern Wisconsin (Fig. 1B). These stands overlaid regions with known or anecdotal *Ixodes* and *Borrelia* incidence. Stands were either symptomatic of Red Pine Pocket Decline (hereafter called diseased) ($n = 21$) or healthy, asymptomatic control stands ($n = 10$). Soils were generally sandy with suboptimal nutrition for *P. resinosa* (Appendix A).

2.2. Tick sampling

We sampled ticks in June and August 2008, and June, August, and October 2009. All stands were sampled within 3 consecutive days on each occasion. In diseased stands, *I. scapularis* were sampled along the edge of tree mortality ("inside" ring) and 10 m outside the edge in apparently healthy forest ("outside" ring). In asymptomatic control stands, the inside ring was 25 m outside a permanently marked central point, and the outside ring was 35 m from the central point. *Ixodes scapularis* were sampled using a standard white flannel sheet dragging technique. Each sheet was

Table 1
Location, type, and size of red pine plantations in Wisconsin where tick and vegetation sampling occurred in 2008 and 2009. Diseased stands with an asterisk received a 1 m deep root-severing treatment 10 m outside the gap edge to stop disease progression; all diseased stands were combined in the final analyses.

Region	County	GPS location	Type	Site size (m^2)
West Salem	Jackson	N 44.25664°, W 90.59812°	Asymptomatic	3851
West Salem	Jackson	N 44.23290°, W 90.66914°	Asymptomatic	3851
West Salem	Jackson	N 44.32760°, W 90.77803°	Asymptomatic	3851
West Salem	La Crosse	N 44.06296°, W 91.07220°	Diseased	1790
West Salem	La Crosse	N 44.05685°, W 91.06637°	Diseased	1696
West Salem	La Crosse	N 44.05636°, W 91.06554°	Diseased	2409
West Salem	La Crosse	N 44.05947°, W 91.06700°	Diseased*	7896
West Salem	La Crosse	N 44.05881°, W 91.06668°	Diseased*	8149
West Salem	La Crosse	N 44.05951°, W 91.07097°	Diseased*	4210
West Salem	La Crosse	N 44.06804°, W 91.07624°	Diseased*	3509
West Salem	La Crosse	N 44.06717°, W 91.07790°	Diseased*	6785
West Salem	La Crosse	N 44.06605°, W 91.07506°	Diseased*	24,512
West Salem	La Crosse	N 44.06526°, W 91.07562°	Diseased*	7162
West Salem	La Crosse	N 44.05982°, W 91.07383°	Diseased*	2300
Central Sands	Sauk	N 43.52845°, W 89.79945°	Asymptomatic	3851
Central Sands	Waupaca	N 44.25253°, W 89.18178°	Asymptomatic	3851
Central Sands	Waupaca	N 44.25128°, W 89.18150°	Diseased	4974
Central Sands	Waupaca	N 44.31342°, W 89.10396°	Asymptomatic	3851
Central Sands	Waupaca	N 44.31124°, W 89.10206°	Diseased	796
Central Sands	Waupaca	N 44.31157°, W 89.10058°	Diseased*	3088
Central Sands	Waushara	N 44.13564°, W 89.24595°	Asymptomatic	3851
Central Sands	Waushara	N 44.13235°, W 89.23622°	Diseased*	2843
Spring Green	Sauk	N 43.18050°, W 90.15342°	Diseased	2813
Spring Green	Sauk	N 43.18067°, W 90.15676°	Diseased*	2166
Spring Green	Sauk	N 43.18861°, W 90.16261°	Asymptomatic	3851
Kettle Moraine	Walworth	N 42.83011°, W 88.61018°	Asymptomatic	3851
Kettle Moraine	Walworth	N 42.82595°, W 88.60961°	Diseased	4584
Kettle Moraine	Walworth	N 42.82797°, W 88.61039°	Diseased*	10,953
Kettle Moraine	Waukesha	N 42.85956°, W 88.49636°	Diseased	4210
Kettle Moraine	Waukesha	N 42.91027°, W 88.46485°	Asymptomatic	3851
Kettle Moraine	Waukesha	N 42.91400°, W 88.46814°	Diseased*	5974

1 m^2, and all drags took place between 0600 and 1300. All ticks were collected from sheets after every 50 m long drag, and gaps ranged in size from <2 to >13 drags in length. Ticks were brought alive to the Medical Entomology Laboratory in the Department of Entomology at the University of Wisconsin, Madison. All ticks were identified to species, and all *I. scapularis* nymphs were tested for the presence of *B. burgdorferi* and *A. phagocytophilum*.

2.3. DNA extraction

Nymphal ticks were placed into 1.7 ml centrifuge tubes and bisected using a sterile 18-gauge hypodermic needle. DNA extraction was performed using a DNeasy blood and tissue kit (Qiagen Inc., Valencia, CA, USA) and the Animal Tissue (Spin-Column) protocol following the manufacturer's instructions. The resulting DNA was aliquoted into two portions with part of the sample stored at −80 °C for future studies and the remainder stored at −20 °C for immediate use in diagnostic PCR assays.

2.4. PCR analysis

DNA extraction, PCR amplification, gel electrophoresis and gel visualization were conducted in three separate rooms and with dedicated pipettes and aerosol-resistant filter pipette tips to reduce the risk of contamination. Each PCR reaction contained 10 μL of EconoTaq Plus Green 2X Master mix (Lucigen Corp., Middleton, WI, USA), 7 μL of nuclease free water, 1 μL of each primer pair and 1 μL of the template DNA. At least two negative controls containing nuclease free water in place of DNA were included in each PCR set. Reaction products were separated by gel electrophoresis in 1% agarose gels in 1 X TAE buffer and gels were stained with ethidium bromide prior to visualization with UV transillumination.

2.5. Detection of B. burgdorferi

A nested PCR assay was performed on nymphal ticks. Oligonucleotide primer sequences used to detect the presence of the *B. burgdorferi* bacteria were primer pair FOspB-1 (GGTGCTGAGTCAATTGGTTCT)/ROspB-2 (TTCTAGGCTGGTTCCAGCTGT) for the primary reaction, and OspB-3 (TTTTCCGACTACAAGACTTCC)/OspB4 (TTAGAAGCATTTGATGCCAGC) for the nested reaction targeting a 400 bp fragment of the OspA/OspB operon gene segment. One microlitre of the primary reaction was used as a template for the nested PCR reaction. The cycling conditions were: initial denaturation at 94 °C for 30 s followed by 30 cycles of denaturation at 93 °C for 30 s, annealing for 1 min at 52 °C, and extension at

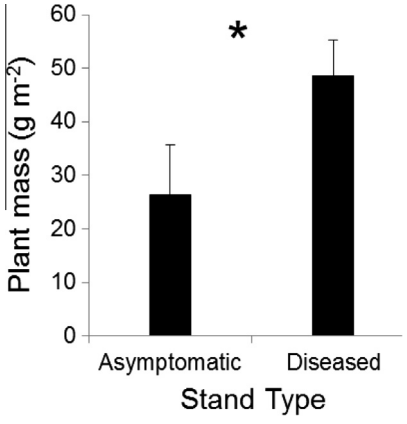

Fig. 2. Understory plant mass in asymptomatic and diseased *P. resinosa* stands in central and southern Wisconsin in fall 2009. The stand health type is noted along the *x*-axis. The amount of dry plant mass collected is shown on the *y*-axis.

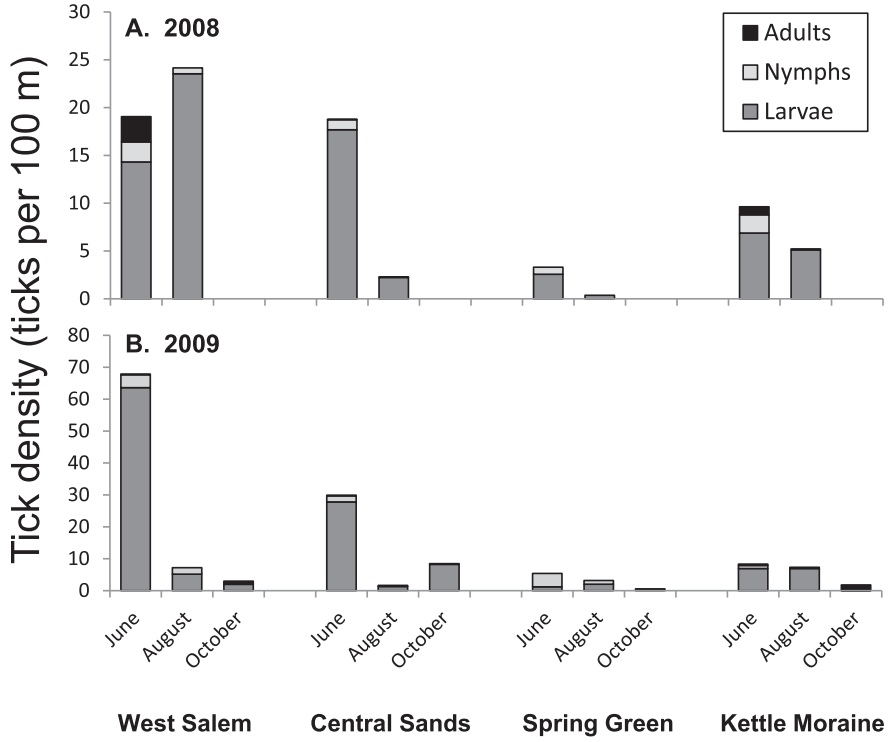

Fig. 3. *Ixodes scapularis* density (mean ± SE) in four study areas in Wisconsin in June and August, 2008 (A) and June, August, and October, 2009 (B). All analyses were performed using SAS (SAS Institute, Cary, NC, USA) with an α-level of 0.05 considered significant.

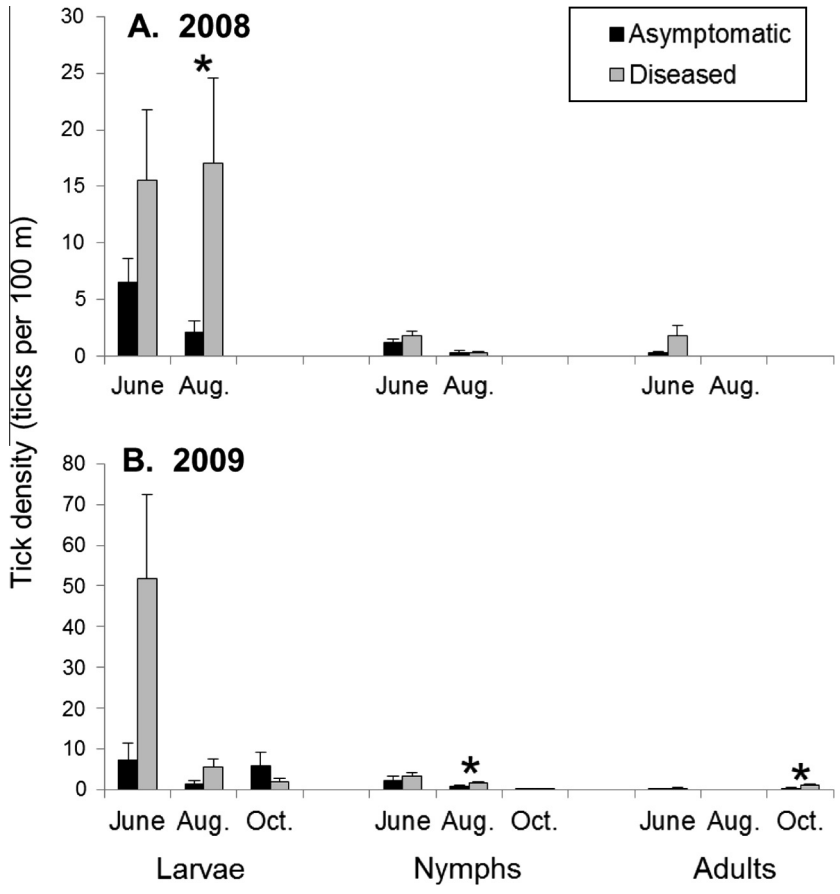

Fig. 4. Tick density (mean ± SE) in asymptomatic and diseased *P. resinosa* stands in 2008 (A) and 2009 (B). The collection month and tick life stage are noted along the *x*-axis. The density of each tick life stage is shown on the *y*-axis (note different *y*-axis scales). Within each month and year, significant differences in tick densities between asymptomatic and diseased stands are indicated with an asterisk. Sampling was not conducted in October 2008.

72 °C for 2 min. A final extension at 72 °C for 10 min was performed (Caporale and Kocher, 1994). The same cycling conditions were used for both the primary and nested reactions.

2.6. Detection of phagocytophilum

A nested PCR using the primer pair ge31/ge10r for the primary reaction and the primer pair ge9f/ge2 for the nested reaction targeting a 546 bp fragment of the 16S rRNA gene was performed for detection of *A. phagocytophilum* (Massung et al., 1998; Steiner et al., 2006). After an initial denaturation at 95 °C for 1 min, 35 cycles of the following were performed: denaturation at 94 °C for 15 s; annealing for 15 s at 68 °C (cycles 1–3), 64 °C (cycles 4–6), 60 °C (cycles 7–9), and 56 °C (cycles 10–35); and extension at 72 °C for 20 s. Additionally, a final extension at 72 °C for 5 min was performed.

2.7. Vegetation sampling

We sampled vegetation from each stand in September 2009. On the inside (diseased) ring and outside (healthy) ring of each diseased and asymptomatic control stand we placed a 1 m^2 quadrat on the east, west, north, and south point. All live vegetation in the understory was clipped at the ground, dried at 60 °C, and weighed to the nearest g.

2.8. Statistical analyses

We analyzed the mean of the four plant biomass samples from each ring (inside or outside). Plant biomass data were log-transformed prior to analysis, and were examined using a generalized linear mixed-effects model that treated forest stand type (diseased or asymptomatic) and the location within a forest stand type (inside or outside) as fixed effects (Littell et al., 2006). Our analysis explicitly accommodated the split-plot structure of our experimental design, as location within a forest stand type represented a treatment applied at a different level of experimental unit than the forest stand type treatment.

Very few *Dermacentor variabilis* were captured (Appendix B), and it is not an important human disease vector in Wisconsin, so this species was excluded from analyses. We examined the abundance of *I. scapularis* using generalized linear mixed-effects models (Littell et al., 2006) that treated forest stand type and the location within a forest stand type as fixed effects, and utilized a Poisson response distribution. Stand location and region were considered random effects. Like the vegetation analyses, our analyses of *I. scapularis* abundance also explicitly incorporated the split-plot nature of our experimental design. To accommodate covariance among 50 m transect segments along the same transect, we explicitly modeled covariance among transect segments within the same transects using a repeated-measures approach. Models using several candidate covariance structures were examined (first-order autoregressive, first order autoregressive moving average, compound symmetry, spatial power, and variance components). We selected a variance components covariance structure for all final models based on comparison of AIC values among candidate models. We used the Kenward–Rogers approximation for estimation of denominator degrees of freedom for tests of fixed effects (Littell et al., 2006).

A second analytical method – logistic regression, using the proportion of drags with presence or absence of ticks – was also used to compare the probability of capturing an *I. scapularis* tick in a particular location. This method explicitly focuses on the effect that stand health has on the chance that an *I. scapularis* is present, although a criticism of this method is that it gives disproportionate weight to rare events (e.g., capturing only one tick on a drag).

The proportion of *I. scapularis* nymphs that were infected with *Anaplasma* or *Borrelia* was examined using generalized linear mixed models that employed a binomial response distribution and the same model structure as our analyses of tick abundance. Because infection rates were generally low, sparse data required pooling among months and examination of only main effects for the examination of *Anaplasma* prevalence. Similarly, *Borrelia* prevalence could only be examined using pooled months or by removing the interaction term from the model. All analyses were performed using SAS (SAS Institute, Cary, NC, USA), and an α-level of 0.05 was considered significant.

3. Results

3.1. Plant biomass was greater in diseased stands

There was twice as much plant biomass in diseased stands than in asymptomatic stands ($F_{1,8.33} = 9.35$, $P = 0.015$) (Fig. 2). Plant biomass was not affected by location within a forest stand type (inside

Table 2
Effects of treatment and stand location on *I. scapularis* density in 2008 and 2009 in central and southern Wisconsin, USA. Sampling was not conducted in October 2008.

Year	Month	Life stage	Effect	*F*	*P*
2008	June	Larvae	Treatment[a]	0.53	0.471
			Ring[b]	0.74	0.390
			Trt × Ring	0.80	0.372
		Nymphs	Treatment	2.03	0.165
			Ring	1.00	0.319
			Trt × Ring	0.06	0.803
		Adults	Treatment	0.79	0.381
			Ring	0.06	0.804
			Trt × Ring	1.32	0.252
	August	Larvae	Treatment	4.23	**0.044**
			Ring	1.05	0.306
			Trt × Ring	2.39	0.124
		Nymphs	Treatment	2.46	0.131
			Ring	0.01	0.911
			Trt × Ring	0.02	0.878
		Adults	Treatment	na[c]	na
			Ring	na	na
			Trt × Ring	na	na
2009	June	Larvae	Treatment	2.40	0.127
			Ring	2.67	0.104
			Trt × Ring	2.27	0.134
		Nymphs	Treatment	2.01	0.167
			Ring	1.65	0.201
			Trt × Ring	0.35	0.557
		Adults	Treatment	1.94	0.168
			Ring	0.02	0.885
			Trt × Ring	0.09	0.765
	August	Larvae	Treatment	2.96	0.086
			Ring	0.65	0.420
			Trt × Ring	1.45	0.230
		Nymphs	Treatment	4.82	**0.033**
			Ring	4.03	**0.046**
			Trt × Ring	0.06	0.815
		Adults	Treatment	na	na
			Ring	na	na
			Trt × Ring	na	na
	October	Larvae	Treatment	0.17	0.686
			Ring	23.48	**<0.0001**
			Trt × Ring	0.00	0.963
		Nymphs	Treatment	na	na
			Ring	na	na
			Trt × Ring	na	na
		Adults	Treatment	11.60	**0.001**
			Ring	1.94	0.165
			Trt × Ring	0.06	0.805

[a] Symptomatic or asymptomatic stand condition.
[b] Location within a forest stand type (inside or outside ring).
[c] No ticks collected.

vs. outside ring; $F_{1,27.82} = 0.54$, $P = 0.470$), nor by the stand treatment × location within a forest stand type interaction ($F_{1,27.82} = 0.73$, $P = 0.400$).

3.2. Greater density and chance of encountering Ixodes ticks in diseased stands

Ixodes scapularis abundance exhibited considerable spatial variation among regions (Fig. 3). Overall, differences associated with plant community structure were in the direction of more ticks in the diseased stands. *Ixodes scapularis* larval density was between 139% and 716% greater in diseased stands during every sampling period except October 2009, when more larvae were captured in asymptomatic stands (Fig. 4, Table 2). Density of *I. scapularis* nymphs was nearly equal in diseased and asympomatic stands in August 2008, but ranged from 43% to 133% greater in diseased compared to asymptomatic stands during the other sampling periods (Fig. 4). Adult *I. scapularis* densities were between 220% and 503% greater in diseased stands (Fig. 4). Within diseased stands, *I. scapularis* densities did not differ between the diseased (inside) and nonsymptomatic (outside) rings. There were no interactions between stand condition (diseased or asymptomatic) and location within a forest stand (Table 2). These results show an increased abundance of ticks in diseased stands.

Our logistic regression analysis showed that a greater proportion of drags were positive for *I. scapularis* larvae and nymphs in diseased than asymptomatic stands in August 2009 (Fig. 5, Table 3). Likewise, adult *I. scapularis* were captured more often on drags in diseased than asymptomatic stands in October 2009. Within diseased stands, the proportion of drags with *I. scapularis* was usually consistent between locations within a forest stand. There was one case where the interaction between stand condition (diseased or asymptomatic) and location within a forest stand (inside or outside ring) affected nymph occurrence – nymphs were more often captured on the outside of diseased stands in June 2008 (Table 3).

3.3. Pathogen infection rates were the same regardless of tick origin

Infection rates with *B. burgdorferi* and *A. phagocytophilum* did not differ between ticks collected from diseased or asymptomatic stands (Bb: $F_{1,33.68} = 0.67$, $P = 0.419$; Ap: $F_{1,12.18} = 0.55$, $P = 0.471$) (Fig. 6) or location within a forest stand (Bb: $F_{1,165.7} = 2.02$, $P = 0.157$; Ap: $F_{1,128.2} = 0.00$, $P = 0.988$). There was no interaction between stand type and location within a forest stand for *B. burgdorferi* ($F_{1,162.4} = 1.04$, $P = 0.309$). *Borrelia burgdorferi* was over five and nine times more common than *A. phagoctophilum* in diseased and asymptomatic stands, respectively (Fig. 6).

4. Discussion

These results illustrate a mechanism by which belowground herbivory can initiate a cascade that ultimately increases population densities of an arthropod vector of a human disease pathogen. The susceptibility to, and feedbacks among, these insect herbivores and their phytopathogenic fungal symbionts are amplified by the structure of conifer plantations. The resulting gaps and early-succession vegetation constitute a habitat that is known to be more favorable to deer and rodents than the more closed canopies of healthy plantations or native forests (Côté et al., 2004; Allan et al., 2010). The role of increased mammal activity is supported

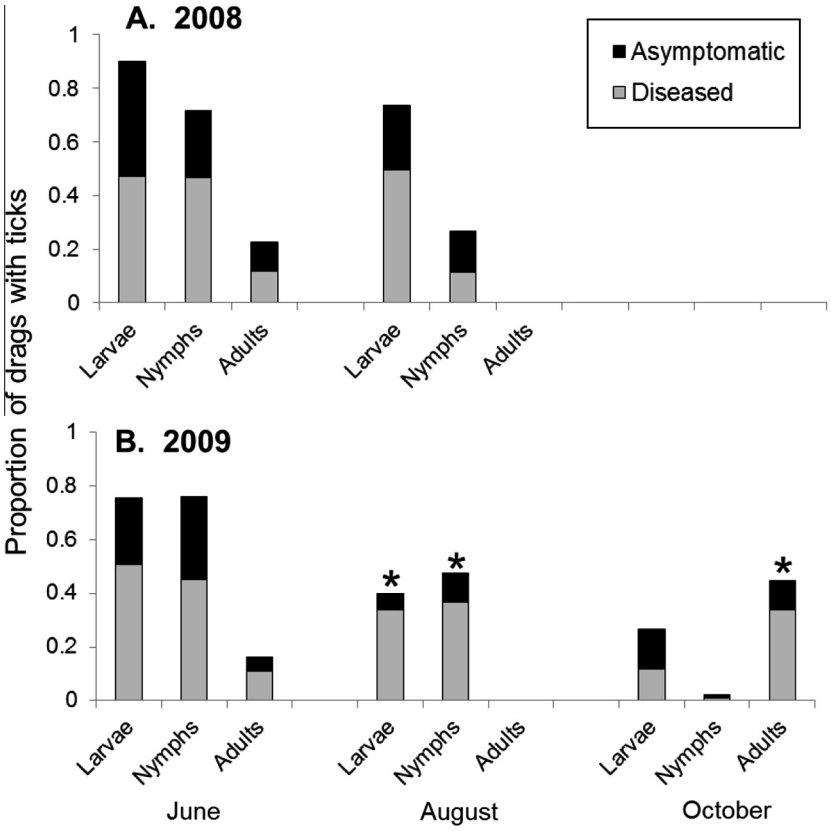

Fig. 5. Proportion of drags capturing *I. scapularis* in asymptomatic and diseased *P. resinosa* stands in 2008 (A) and 2009 (B). The proportion of drags having a tick life stage present is shown on the *y*-axis. The tick life stage and collection month are shown along the *x*-axis. Significant differences in the proportion of drags with ticks between asymptomatic and diseased stands are indicated with an asterisk. Sampling was not conducted in October 2008.

Table 3
Statistical analysis of the proportion of drags collecting *I. scapularis* at 31 sites in Wisconsin in 2008 and 2009. Sampling was not conducted in October 2008.

Year	Month	Life stage	Location	*F*	*P*
2008	June	Larvae	Treatment[a]	0.00	0.979
			Ring[b]	0.54	0.465
			Trt × Ring	3.48	0.064
		Nymphs	Treatment	2.96	0.100
			Ring	0.32	0.572
			Trt × Ring	4.54	**0.035**
		Adults	Treatment	0.03	0.858
			Ring	9.05	**0.003**
			Trt × Ring	0.22	0.636
	August	Larvae	Treatment	2.26	0.149
			Ring	1.42	0.235
			Trt × Ring	0.06	0.814
		Nymphs	Treatment	2.25	0.151
			Ring	0.33	0.568
			Trt × Ring	0.68	0.409
		Adults	Treatment	na[c]	na
			Ring	na	na
			Trt × Ring	na	na
2009	June	Larvae	Treatment	2.84	0.108
			Ring	12.89	**0.0004**
			Trt × Ring	0.28	0.597
		Nymphs	Treatment	0.63	0.437
			Ring	0.22	0.641
			Trt × Ring	0.01	0.923
		Adults	Treatment	3.55	0.063
			Ring	0.74	0.390
			Trt × Ring	0.09	0.768
	August	Larvae	Treatment	7.32	**0.009**
			Ring	2.90	0.090
			Trt × Ring	1.11	0.293
		Nymphs	Treatment	5.84	**0.022**
			Ring	3.06	0.082
			Trt × Ring	0.05	0.832
		Adults	Treatment	na	na
			Ring	na	na
			Trt × Ring	na	na
	October	Larvae	Treatment	0.39	0.541
			Ring	2.70	0.102
			Trt × Ring	0.00	0.958
		Nymphs	Treatment	na	na
			Ring	na	na
			Trt × Ring	na	na
		Adults	Treatment	7.92	**0.007**
			Ring	4.61	**0.033**
			Trt × Ring	0.78	0.380

[a] Symptomatic or asymptomatic stand condition.
[b] Location within a forest stand type (inside or outside ring).
[c] No ticks collected.

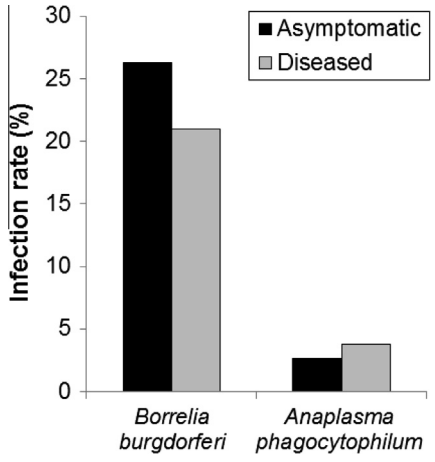

Fig. 6. *Borrelia burgdorferi* and *A. phagocytophilum* infection rates in *I. scapularis* nymphs captured in 2009. Infection rates did not differ between asymptomatic and diseased stands.

by the stand-level, rather than within-stand level, nature of the response, which is more indicative of the range of mammalian than *Ixodes* movement. The effect of this cascade appears to arise from increased *I. scapularis* abundance, rather than increased infection rates with *B. burgdorferi* or *A. phagocytophilum*, or from increases in other tick species. The vegetation structure that arises from this interaction between below- and above- ground herbivores, and that supports higher tick and potentially higher rodent populations, is unknown, but appears to be fairly long-lived. For example, in a long-term study we reported gap proliferation still continuing after 15 years of annual observation, and showed no canopy closure (e.g., no deciduous trees were >9.0 cm DBH) at 10 years (Aukema et al., 2010). Similarly, the gaps in our diseased stands ranged from 796 to 24,512 m^2, indicating an extensive chronosequence for a syndrome that extends only several meters per year (Erbilgin and Raffa, 2003; Aukema et al., 2010).

Exotic plants, pathogens, and insects can affect tick densities by altering ground microclimate (Civitello et al., 2008; Williams and Ward, 2010) and favoring host populations (Jones et al., 1998; Williams et al., 2009; Allan et al., 2010; Swei et al., 2012). Our work suggests that forestry practices, by favoring native herbivores, can likewise increase human disease risk. Four features of conifer plantations are particularly conducive to management of the dynamics we observed: (1) The proximity of hosts favors *Hylobius* weevils that infest roots and vector *Leptographium*, because they disperse largely by walking in Wisconsin (Rieske and Raffa, 1990); (2) Extensive root grafting provides radiating corridors for *Leptographium*; (3) Populations of the major predator of *Dendroctonus* and *Ips* beetles, *Thanasimus dubius* (F.), are reduced (Ryall and Fahrig, 2005), apparently because this predator is less likely than its prey to cross edges and move into newly infested plantations (Costa et al., 2013); (4) Resulting gaps favor subsequent establishment by more virulent phytopathogens that can co-occur with *Leptographium* (Otrosina et al., 1999). The root fungus *Heterobasidion irregulare* became established in 43% of the diseased stands (4.7 ± 0.5 years after the initial survey), whereas only 10% of asymptomatic stands became infected.

Increases in tick populations are influenced by a wide range of natural, anthropogenic, and in this case interacting natural and anthropogenic disturbances, rather than any one syndrome (Jones et al., 1998; Estrada-Peña, 2002; Allan et al., 2010; Swei et al., 2011). Hence we observed high variation at all levels of spatial and temporal scale. Additionally, the clumped spatial distribution of ticks contributed to occasionally high variance among samples. In nearly all cases there was a greater chance of encountering ticks in diseased than healthy stands, but these differences were not consistently statistically significant. Future research is needed to better quantify sources of numerical variation in *Ixodes* populations. Complementing the insights from extensive landscape-scale studies with the intensive site-specific sampling needed to account for local trends remains a major challenge.

Red pine plantations in Wisconsin are often on sites at the southernmost edge of red pine's native range (Little, 1971; Scheller and Mladenoff, 2005) and with marginal soil quality. Trees growing under stressed conditions are more susceptible to bark beetles such as *I. pini* (Say) and *D. valens*, and sandy soils provide the most conducive conditions for root insects such as *Hylobius* spp. (Wilson and Millers, 1982). Soils at our sites were both sandy and outside the optimal nutrition range for red pine (Appendix A). Climate models predict that rising temperatures will further reduce the suitability of this region for red pine (Prasad et al., 2007; Iverson et al., 2008), which will likely amplify the feedbacks we described. Thus, the combined factors of plantation structure and location, anthropogenic climate change, altered microclimate, and invasive pathogens seem likely to jointly favor the local abundance of some arthropod vectors of human disease pathogens.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2013.03.017.

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