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Changes in canopy structure and ant assemblages affect soil ecosystem variables as a

foundation species declines

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Abstract.   The decline of *Tsuga canadensis* (eastern hemlock) – a foundation tree species – due to infestation by *Adelges tsugae* (hemlock woolly adelgid) or its complete removal from a stand by salvage logging dramatically affects associated faunal assemblages. Among these assemblages, species composition (richness and abundance) of ants increases rapidly as *T. canadensis* is lost from the stands. Because ants live and forage at the litter-soil interface, we hypothesized that environmental changes caused by hemlock loss (e.g., increased light and warmth at the forest floor, increased soil pH) and shifts in ant species composition would interact to alter soil ecosystem variables. In the Harvard Forest Hemlock Removal Experiment (HF-HeRE), established in 2003, *T. canadensis* in large plots were killed in place or logged and removed to mimic adelgid infestation or salvage harvesting, respectively. In 2006, we built ant exclosure subplots within all of the canopy-manipulation plots to examine direct and interactive effects of canopy change and ant assemblage composition on soil and litter variables. Throughout HF-HeRE, *T. canadensis* was colonized by the adelgid in 2009, and the infested trees are now declining. The experimental removal of *T. canadensis* from the canopy was associated with an increase in the rate of cellulose decomposition by >50%, and exclosure of ants from subplots directly reduced their soil nitrate availability by 56%. Partial least squares path models revealed sequential interactive effects prior to adelgid infestation: canopy change (as a proxy for associated environmental changes) altered both decomposition and ant assemblage structure; changes in ant assemblage structure and decomposition rates altered nitrogen availability. The results illustrate that biotic changes directly associated with decline of *T. canadensis* can have cascading effects on ecosystem nutrient availability and cycling.

Key words: *Adelges tsugae*; ecosystem dynamics; foundation species; Formicidae; Harvard Forest; indirect effects; nitrogen; soil respiration; *Tsuga canadensis*.

INTRODUCTION

Global redistribution of species and reorganization of species assemblages associated with climatic changes and human activities (e.g., Garcia et al. 2014) are having widespread effects on the composition and function of ecosystems (reviewed in Bardgett and Wardle 2010). These effects can be particularly large when a foundation species (sensu Dayton 1972) declines or is removed, because these dominant, widespread species play disproportionate roles in structuring ecological systems (reviewed by Ellison et al. 2005b). In particular, foundation species often control the distribution and abundance of many associated species via non-trophic interactions (Baiser et al. 2013).

Tsuga canadensis (L.) Carrière (eastern hemlock) is a foundation tree species in eastern North American forests (reviewed in Ellison and Baiser 2014). Populations of this species are declining due to infestation by a rapidly spreading nonnative insect, *Adelges tsugae* Annand (hemlock woolly adelgid) (Orwig et al. 2008). In addition, many eastern hemlock stands are being pre-emptively cut in expectation of future adelgid infestations, so as to salvage merchantable timber (Foster and Orwig 2006). Composition and abundance of many associated plant and animal species, including understory herbs (Orwig et al. 2013), insects (Ellison et al. 2005a, Dilling et al. 2007, Rohr et al. 2009, Sackett et al. 2011), spiders (Sackett et al. 2011), salamanders (Mathewson 2009, 2014), and birds (Tingley et al. 2002, Brown and Weinka 2014), change following decline and subsequent loss of *T. canadensis* due to the adelgid or its rapid removal by logging.

Notably, ant species richness and abundance increase rapidly following either mechanism of *T. canadensis* loss (Ellison et al. 2005a, Rohr et al. 2009, Sackett et al. 2011) as the forest floor is warmed (Lustenhouwer et al. 2012) and soil pH changes. Because ants are herbivores,

seed harvesters, omnivorous predators, and soil and litter movers, changes in their diversity and abundance also can alter ecosystem structure and function (reviewed by Del Toro et al. 2012). Foundation species also modulate ecosystem processes such as carbon and water flux, decomposition, and nutrient cycling (e.g. Ellison et al. 2005b, Ford and Vose 2007). Changes to forest ecosystem dynamics following pre-emptive salvage logging are similar in scale, but occur more rapidly than changes seen following adelgid infestation (Orwig et al. 2013).

We hypothesized that direct effects of the loss of a hemlock canopy, subsequent effects of changes in ant diversity and abundance associated with hemlock decline, and the interactions of changes in canopy structure and ant assemblage composition, together would lead to a cascade of changes in litter, soil, and vegetation dynamics in declining hemlock stands. To test this hypothesis, we manipulated ant abundance within the Harvard Forest Hemlock Removal Experiment (HF-HeRE), a long term, multi-hectare-scale, canopy-manipulation experiment designed to study the effects of hemlock loss on northeastern USA forests (Ellison et al. 2010, Orwig et al. 2013). We directly measured effects of changes in ant abundance on the structure of understory plant assemblages and on soil-level ecosystem variables that link the litter and soils in which ants live – cellulose and lignin decomposition, soil nitrogen availability, and soil respiration – and used path analysis to explore causal linkages between forest canopy structure, hemlock decline, ant assemblages, and soil ecosystem variables. We also took advantage of the design of HF-HeRE to contrast how these effects differed among stands where *T. canadensis* was killed instantly by logging or girdling versus stands where *T. canadensis* is declining much more slowly as the adelgid colonizes them.

METHODS

Study site and canopy treatments

HF-HeRE is sited in Petersham, Massachusetts, within the 121-ha Simes tract at the

Harvard Forest (42.47 – 42.48°N, 72.22 – 72.21°W; 215 – 300 m a.s.l). The tract comprises large

areas dominated by *T. canadensis* throughout; other common trees include red maple (*Acer*

rubrum L.), red oak (*Quercus rubra* L.), black birch (*Betula lenta* L.), and white pine (*Pinus*

strobus L.) (Ellison et al. 2010, 2014). The current dominance of *T. canadensis* at the Simes tract

is relatively recent, and resulted from competitive release and rapid growth following loss of

American chestnut (*Castanea dentata* (Marsh.) Borkh.) in the early 1900s, logging of pines from

the tract during the 1880s, 1920s, and 1930s, and damage to pines and hardwoods during the

1938 "Great Hurricane" (Ellison et al. 2014).

A full description of HF-HeRE is in Ellison et al. (2010); only pertinent details are

mentioned here. The experiment, established in 2003, consists of eight ≈90×90-m (0.81 ha) plots,

divided into two blocks. The four plots in the "valley" block are situated in the central valley of

the tract, whereas those on the "ridge" block are ≈500 m northeast of the valley plots. The valley

block is characterized by poorly drained, swampy soils. Besides *T. canadensis*, *A. rubrum, P.*

strobus, and mixed hardwoods are common in the valley block. Soils in the ridge block are better

drained, and this area is dominated by *Q. rubra, Q. alba* L., and *P. strobus*, in addition to *T.*

canadensis.

Within each block, three hemlock-dominated (>65% of the canopy and basal area) plots

and one hardwood plot were sited at the beginning of the experiment. Of the three hemlock-

dominated plots, one was left untreated as a "hemlock control" while the other two were

manipulated. In the "girdled" plots, the bark and cambium of every individual hemlock stem

were cut through in May 2005 to simulate rapid decline and tree death associated with adelgid

infestation (Yorks et al. 2003). In the "logged" plots all hemlocks >20 cm DBH, 50% of

merchantable oak and pine, and some poor-quality red maple and birch (for firewood) were

felled and removed between February and April 2005 to mimic standard silvicultural practices

used to salvage the value of the timber before the adelgid arrives (Foster and Orwig 2006).

Because of the large size of the plots and the aspect of the blocks, plots were not randomly

arrayed. Rather, to avoid shading manipulated plots in each block by the associated hemlock

control plot, logged plots were always sited south of hemlock control plots, and girdled plots

were in between the logged and hemlock control plots. Plots were separated from one another by

5–10 m. The fourth plot in each block was a "hardwood control" plot dominated by young

hardwoods, and which represented the expected near-term (20–50 year) structure of forests from

which hemlock has been lost (i.e., a space-for-time substitution) (Albani et al. 2010). In each

block, the hardwood control was located >100 m from the three hemlock plots. Together, these

four plot types are intended to serve as a short-term chronosequence of forests invaded by the

adelgid; hemlock control plots represent hemlock stands pre-invasion, girdled and logged plots

represent the immediate effects of infestation and salvage logging respectively, and hardwood

control plots represent the eventual future of invaded stands.

Technically, HF-HeRE is a modified Before-After-Control-Impact experiment that was

designed with three distinct temporal phases (Ellison et al. 2010). The "Before" phase ran from

2003−2005. During those two years, baseline data were collected on all plots. The "After-but-

pre-adelgid" phase ran from 2005−2009. At the beginning of this phase (spring 2005), the

adelgid had not yet colonized these plots (nor had it yet been found anywhere in Petersham

outside of a single cultivated hemlock tree near the town's northern border). Between the spring

of 2005, when the two canopy-manipulation treatments – logging or girdling – were applied to individual plots in each block, and mid-2009, when the adelgid was first detected in large numbers (occurrences on trees) at HF-HeRE, the focus of the experiment was on contrasting forest ecology and soil ecosystem variables between intact *T. canadensis* stands (the hemlock controls) and stands in which *T. canadensis* had been removed (by logging) or was simply falling apart (following girdling). The "After-and-post-adelgid" phase began in 2010. Once the adelgid began to irrupt in the hemlock control plots, the experimental contrast of interest shifted to that between stands in which *T. canadensis* had disintegrated due to physical damage alone (girdling) versus stands in which *T. canadensis* was disintegrating due to the adelgid (see Orwig et al. 2013). Thus, since 2010, we have assessed additive and interactive effects on forest dynamics due to physical loss of hemlock and presence of the adelgid (see also Stadler et al. 2005, 2006).

Light environment

Because the dense canopy of *T. canadensis* creates deep shade on the forest floor (Lustenhouwer et al. 2012), changes in the amount of light reaching the forest floor is one of the best measures of decline or removal of hemlock from a forest stand. Hemispherical canopy photographs have been taken twice yearly in each of the HF-HeRE plots since 2005: in April before leaf bud-break by the deciduous trees ("leaf-off" condition), and in September, before the deciduous trees have dropped their leaves ("leaf-on" condition). Twenty-five photographs were taken in each plot on a 15-m grid (technical details in Lustenhouwer et al. 2012). From each photograph, we calculated the global site factor (GSF), which ranges from 0 to 1 and is the estimated proportion of (direct or diffuse) solar radiation reaching the camera (Rich 1989); here, GSF measures an "ants-eye" view of light available (in the forest understory, 1–2 m above

ground). Initial analysis found no evidence of spatial autocorrelation at the scale of the 15-m

grid, showed that each of the 25 photograph locations within each plot showed similar patterns

over time, and that differences among them (within-plot, at each sample date) were constant (A.

M. Ellison and M. Levine, *unpublished data*). Thus, to compare among the plots, we used the

mean GSF value from all 25 photographs taken in each a plot at each measurement time.

Ant treatments

Within each canopy treatment plot, six 3×3-m ant manipulation subplots were installed in

April 2006. Three subplot types were installed: "control," "disturbance control," and "ant

exclosure." Each subplot type was replicated twice within each canopy treatment plot and was

randomly sited within the 7200 m^2 area of the canopy-manipulation plot outside of the central

30×30-m "core" area (which is otherwise intensively sampled for many other variables: Ellison

et al. 2010) and at least 15 m from the edge of the canopy-manipulation plot. This split-plot

design (small replicate ant manipulation subplots within each large canopy-manipulation plot)

was used to separate direct effects of changes in ant assemblage structure on soil ecosystem

variables from those caused by canopy manipulations and adelgid infestation, which themselves

may affect ant species composition (Ellison et al. 2005a, Sackett et al. 2011).

Ant exclosures followed the design of Wardle et al. (2011); each consisted of an outer

wall of 30-cm high fiberglass roofing buried 10 cm in the ground around the perimeter of the

plot, and an inner wall of 8-inch (≈20-cm) diameter PVC sewer pipe cut lengthwise and buried

5-cm deep around a 2×2-m square, inside of which all measurements were taken. Exclosure

walls were coated with Tanglefoot aerosol spray (Biocontrol Network LLC, Brentwood,

Tennessee) in May and July each year. Control subplots were flagged at the corners but were

otherwise unmanipulated, whereas disturbance control subplots were trenched and cleared of rocks around the edges in the same manner as the ant exclosures to control for possible confounding effects of the soil disturbance associated with exclosure installation. As with the exclosure plots, all measurements in the controls and disturbance controls were made within a central 2×2-m area.

In each of the ant manipulation and associated control subplots, two 7-cm diameter × 10-cm deep, 230-ml plastic cups ("pitfall traps") were buried flush with the soil surface. From 2006 through 2014, three times each summer, traps were opened, partially filled (20 ml) with a mixture of water and a few drops of dishwashing detergent, and then left uncovered for 48 hours, after which time all ants in the pitfalls were removed, counted, and identified to species following Ellison et al. (2012). Voucher specimens are deposited in Harvard University's Museum of Comparative Zoology. In addition to supplying necessary data on ant assemblages that could be associated with changes in soil ecosystem variables, data from the control pitfalls were used to test for differences in ant assemblage composition among the canopy treatment plots, and data from the exclosures were used to verify that the exclosures effectively reduced ant activity.

Vegetation

Understory vegetation, including herbs, tree seedlings and suppressed saplings, responds rapidly to the increasing light availability attendant to hemlock decline (Catovsky and Bazzaz 2000, Orwig et al. 2013), and many understory species are dispersed by ants (Stuble et al. 2014). Understory vegetation (plants < 1.3 m tall and <1.0 cm diameter) was sampled annually in the canopy-manipulation plots in five 1-m^2 plots spaced evenly along each of two 30-m transects

(for herbs and seedlings) and within the central 30×30-m "core" area (for saplings) of each canopy removal plot (Orwig et al. 2013). Identity and abundance of herbs, shrubs, and tree seedlings and saplings were recorded in these annual samples. Vegetation within the ant-manipulation subplots was assessed in July 2014. For most species, we counted individual herbs, shrubs, and tree seedlings and saplings; percent cover was estimated for graminoids and herbs, such as *Mitchella repens* L., that form large, spreading clones. Basal area was computed from measurements of diameter at breast height (DBH, measured at 1.3 m height) of tree saplings > 1 cm DBH.

Soil ecosystem variables

Soil NO_3^- (nitrate) and NH_4^+ (ammonium) availability in the ant manipulation subplots were measured quarterly from July 2006 through October 2014. A single home-built WECSA-type (WECSA, LLC, Saint Ignatius, Montana, USA) soil-access tube constructed of a 1½" (≈4 cm) diameter PVC outer sleeve and a 1" (≈2.5 cm) diameter removable PVC inner sleeve was installed at an ≈45° angle into the mineral soil (≈15 cm depth) at a random location within each subplot. A Unibest PST-1 resin capsule (WECSA, LLC) was placed at the end of the inner sleeve and in contact with the soil. Resin capsules were collected and replaced every three months, extracted with 60 ml of 1M KCl solution, and analyzed for NO_3^- and NH_4^+ content using a Lachat auto-analyzer (analytical details in Orwig et al. 2013).

A single 30-cm diameter, 10-cm high PVC soil respiration collar was partially buried (5-cm deep) at a random location in each subplot in May 2006, and soil respiration was measured in these collars biweekly during the summers of 2006, 2007, and 2008 using a LI-COR 6200 portable IRGA (LI-COR Inc., Lincoln, Nebraska; detailed methods in Giasson et al. 2013).

Fourteen of these biweekly "runs" were completed over the three years (three in 2006, four in 2007, and seven in 2008); in each run, every subplot was measured within 5 days. Because soil temperatures were measured continuously only at the scale of the canopy-manipulation plot (Lustenhouwer et al. 2012), not within each subplot, we could not estimate annual CO_2 flux within ant-manipulation subplots. Rather, we assessed the effects of ant manipulations on instantaneous (within-run) CO_2 fluxes among subplots.

Decomposition was assessed using standard substrates in mesh bags constructed of 3-mm nylon mesh (on the top) and 1.3-mm mesh plastic window screen (on the bottom). Cellulose filter papers (5-g Whatman #1) were placed in 15.5×15.5-cm mesh bags, and lignin tongue depressors (15×2 cm) were placed in 19×5-cm mesh bags. The larger mesh size on top allowed ants and other litter microarthropods to access the cellulose or lignin, while the smaller mesh on the bottom reduced substrate loss due to fragmentation. Bags were stitched together on three sides with polyester thread and closed with stainless steel staples. Decomposition rates were measured in each subplot over 18 months. In September 2008, two mesh bags containing lignin substrates and three mesh bags containing cellulose substrates were placed on the soil surface at random locations in each subplot. One bag containing filter paper was collected from each subplot in December 2008, and bags containing cellulose or lignin (one each) were collected in June 2009 and March 2010. After collection, the contents of each bag were removed, sorted to remove foreign material, dried to constant mass at 70 °C, and weighed (± 0.001 g). Samples were ashed in a muffle furnace at 550 °C for 6 hours; data are presented as ash-free oven-dried mass.

Statistical analysis

The overall experimental design is a split-plot analysis of covariance (ANCOVA) – six ant subplots within each canopy-manipulation plot – within two blocks sampled through time. We recognize that two blocks is a less-than-ideal small sample size, but siting even these two blocks required nearly 10 ha of relatively homogeneous hemlock-dominated forest area. Nonetheless, because $N > 1$, variance can be estimated and used in the ANCOVA. Subplot type entered into the model as a fixed effect nested within canopy treatment (also treated as a fixed effect). Block (ridge, valley) entered as a random effect, and sample year (for ants), month (for soil nitrogen), or day-of-year (for soil respiration) were included as covariates. To avoid pseudoreplication (sensu Hurlbert 1984) among split-plots, data from the two subplots for each treatment were pooled within each canopy treatment plot. Ant data were further pooled by year (three pitfall samples/year), from which we calculated total ant abundance, species richness, and the effective number of ant species (e^H: Chao et al. 2014). Effects of canopy- and ant manipulations on understory plant assemblages were assessed using principal component analysis (PCA). Annual changes in composition of understory vegetation (ferns and fern allies, graminoids, herbs, shrubs) in the different canopy-manipulation plots were visualized as trajectories through principal-component space. The loading scores from that PCA were then applied to the understory vegetation data collected in 2014 in the ant manipulation subplots to predict their location in principal component space. The effects of canopy manipulation, ant manipulation, and their interaction were then tested on the meaningful principal components (as determined from scree plots) for all replicate subplots. Densities of tree saplings in the canopy-manipulation plots and ant manipulation sub-plots were compared using ANOVA.

To examine effects of ant manipulations on three of the soil ecosystem variables – NO_3^- and NH_4^+ availability, and soil respiration – we subtracted the value of the control at each sample

time from the corresponding values in the disturbance control or the exclosure subplots; the expected value of each of these differences under $H_0 = 0$. Decomposition rates of cellulose and lignin were estimated by fitting a linear model to all of the data (proportion of material remaining at each observation period) for each pair of replicate subplots. For cellulose, we included a pre-deployment value of 100%, but because lignin decomposed very slowly over the first 9 months, we modeled only its decomposition over the second 9 months of observations. The estimated slope for each pair of replicate subplots was used as an estimate of the decomposition rate for each treatment. Differences in decomposition rates were analyzed using split-plot ANOVA; no covariate was included because the estimation of rate implicitly accounted for elapsed time.

We used partial least-squares path modeling (plspm) to explore how canopy treatments and ant assemblage structure directly and indirectly influenced the measured soil ecosystem variables. In the path models, we used only the observations made during the time that decomposition rate was assessed (2008-2010); thus, soil respiration data (collected only in 2006-2008) and vegetation data (collected only in July 2014) were not used in the path analysis. The path model assessed: direct effects of canopy treatment on ant assemblages, decomposition rates, and soil nitrogen availability; downstream effects of ant assemblage structure on decomposition and soil nitrogen availability; and finally, effects of lignin and cellulose decomposition on soil nitrogen availability. For consistency, ant assemblage and decomposition variables were transformed to differences from controls prior to path modeling.

Finally, for available data (ant species composition and abundance, light, and soil nitrogen), we contrasted overall (2006−2014) responses with analyses done pre- (2006−2009) and post- (2010−2014) colonization of the adelgid. Theory predicts that during a transition from one state (here, dominated by *T. canadensis*) to another (here, young hardwoods), that variance

in response variables should increase dramatically (so-called critical slowing down: Dakos et al. 2012).

All analyses were done in R version 3.1.2 (R Core Team 2014). ANCOVA models were fit using the aov() function in the stats package; linear models were fit using the lm() function in the stats package and the segmented() function in the segmented package (Muggeo 2014); principal components analysis on scaled and centered data were modeled and fit with prcomp() and predict() in the stats package; and path modeling was done using the pls() function in the plspm package (Sanchez 2013). In the path model, all "inner model" variables other than ant diversity were reflected by a single measured variable; overall ant diversity was considered to be a latent variable reflected by species richness and the effective number of species. Raw data and R code are available from the Harvard Forest Data Archive (http://harvardforest.fas.harvard.edu/data-archive), datasets HF106 (understory vegetation), HF107 (light), and HF-160 (ant collection data and ecosystem measurements within the ant-exclosures and R code for this paper). A knitted html document with model code, full statistical output, and additional diagnostic graphs is provided in the Supplement.

RESULTS

Light and understory vegetation

After treatments were applied, but prior to the colonization of the plots by the adelgid in 2009, light levels were lowest in the hemlock control plots and highest in the logged plots (Fig. 1). The effect of adelgid colonization on light reaching the forest floor was rapid in the hemlock control stands. Before 2009, in both summer (when *Quercus* species [oaks] and other deciduous trees were in full leaf) and winter (when they were not), light levels in the hemlock control plots

were approximately constant at < 10% (Fig. 1). From 2010 through early 2013, however, light levels in the hemlock control plots increased at 3% per year in the winter (as the *T. canadensis* canopy thinned). Since the summer of 2013, however, light levels have stabilized as the deciduous canopies have expanded. Similarly, in the logged plots (in which only *T. canadensis* individuals > 20 cm diameter were removed) and the hardwood control plots (which have scattered *T. canadensis* trees), winter light levels since have been increasing at 4% since 2007 and 6% since 2011, respectively. However, the preponderance of deciduous trees in the hardwood controls, and the rapid growth of deciduous saplings in the logged plots, precluded detection of any light response related to hemlock loss after 2009. There has been no change in summer light levels in the hardwood control plots since 2006, and the dramatic change in light levels in the logged plots occurred three years after the logging, when the saplings exceed 2 m in height (overtopping the camera). Similarly, changes in light levels in the girdled plots were detected after the trees had all died by the end of 2007 (Fig. 1).

The composition of understory vegetation (ferns, herbs, and shrubs) sampled before the canopy treatments in plots that were initially dominated by hemlock had only scattered individuals of a few species (*Mitchella repens*, *Coptis trifolia* (L.) Salisb., *Lysimachia borealis*, and scattered ferns). In contrast, the hardwood controls had an abundant, diverse understory. These differences are apparent in the separation of the hardwood controls from the other plots in principal component space (Fig. 2); all taxa that loaded negatively on both axes (Table 1) were characteristic of hardwood-dominated stands. Over time, the understory vegetation in the girdled and logged plots acquired more species and diverged (in principal component space) from the hemlock controls, but not in any consistent direction (Fig. 2). The composition of understory

vegetation sampled in 2014 in the ant-manipulation subplots was similarly variable (Fig. 2) and did not differ among canopy- ($P = 0.21$, ANOVA) or ant- ($P = 0.79$) manipulation (sub)plots.

Some saplings (tree species for which individuals > 1.3 m tall and < 5 cm DBH) occurred in the understories of the canopy-manipulation plots and within the ant-manipulation subplots. Sapling densities differed among the canopy manipulation plots ($F_{2,10} = 8.8$, $P = 0.006$), being highest in the girdled (10,000 – 15,000/ha) and logged (8,000 – 9,000 /ha) plots, much lower in the hardwood control plots (200 – 500/ha), and absent in the hemlock control plots. *Betula lenta* saplings accounted for > 70% of the saplings in the center of the canopy manipulation plots. Only saplings of this species also were found in the ant-manipulation subplots, where they grew at similar densities to those in the canopy-manipulation plots ($F_{3,10} = 0.65$, $P = 0.6$).

Ants

Nearly 2,500 individual ants representing 32 species and nine genera were collected from pitfall traps over the course of the experiment (Table 2). *Aphaenogaster picea* and *Camponotus pennsylvanicus* were the most common species, together accounting for more than 50% of individuals collected. Other common species included *Formica subsericea, Lasius umbratus* and *Myrmica punctiventris*, each representing more than 100 individuals. No *Formica* species were collected in the hemlock control plots.

Overall, ant abundance and effective number of species all increased significantly between 2006 and 2014 (respectively: $F_{1,191} = 44.5$; $F_{1,191} = 33.0$; all $P < 0.001$; Fig. 3). Within canopy-manipulation plots, ant abundance was significantly lower in the exclosures than in either the controls or disturbance controls (Fig. 4A; $F_{2,8} = 6.26$, $P = 0.02$): 35, 52, 40, and 36% in the hemlock control, girdled, logged, and hardwood control plots, respectively. Ant diversity

(expressed as the effective number of ant species [Fig. 4B]) was highest in the logged and girdled

plots and lowest in the hemlock control plots ($F_{3,3}$ = 15.78, P = 0.02), but did not differ among

the ant exclosure subplots ($F_{2,8}$ = 2.92, P = 0.11). Overall, ant assemblage composition remained

relatively constant over the course of the experiment in both the hemlock and hardwood control

plots, but in the girdled and logged plots, species accumulated through time (Figs. 3, 5; Table 3).

These changes were much more pronounced in the ant control and disturbance-control subplots

than in the ant exclosures (Fig. 5).

Differences in responses of ants to canopy manipulations and subplot treatments also

differed before and after the adelgid colonized HF-HeRE. Through 2009, before the adelgid

colonized these plots, abundance differed among treatments, and was much lower in the ant

exclosures than in either the controls or disturbance controls ($F_{2,8}$ = 7.10, P = 0.017; Fig. 4C).

Prior to the arrival of the adelgid, effective number of species also was lower in the ant

exclosures in all but the logged treatment, though the effect was marginally significant ($F_{2,8}$ =

3.77, P = 0.07; Fig. 4D). After 2010, when the adelgid was present in the plots, effective number

of species differed among canopy-manipulation plots ($F_{3,3}$ = 41.78, P = 0.006), but abundance

did not differ among manipulations, and none of the ant-level response variables differed among

ant manipulation subplots.

Soil ecosystem variables

The decomposition rate of cellulose was fastest in the hardwood plots and slowest in the

logged plots ($F_{3,3}$ = 16.8, P = 0.02; Fig 6A), but there were no significant differences among

canopy treatments in the decomposition rates of lignin ($F_{3,3}$ = 1.63, P = 0.35; Figs. 6B). Ant

treatment had no effect on decomposition rate of either cellulose or lignin ($F_{2,8}$ = 0.42, P = 0.67;

$F_{2,8} = 0.91$, $P = 0.44$, respectively; Figs. 7A, 7B). Soil respiration did not differ significantly

among canopy treatments ($F_{3,3} = 2.69$, $P = 0.22$) or ant treatments ($F_{3,3} = 2.15$, $P = 0.22$),

although it tended to be somewhat higher in the ant exclosures than in the disturbance controls

(Figs. 6C, 7C).

Over the entire experiment, neither soil NH_4^+ nor soil NO_3^- availability differed among

the canopy treatment plots (NH_4^+: $F_{3,3} = 3.22$, $P = 0.18$, Figs. 6D, 7D; NO_3^-: $F_{3,3} = 0.32$, $P =$

0.81; Figs. 6E, 7E). Soil NH_4^+ availability was similar through time (Fig. 6D) and among ant

treatments (Fig. 7D) (respectively: $F_{1,447} = 2.04$, $P = 0.15$, $F_{1,4} = 1.06$, $P = 0.36$; Figs. 6D, 7D). In

contrast, within canopy treatments, NO_3^- availability declined over the course of the experiment

($F_{1,447} = 4.9$, $P = 0.03$; Fig. 6E) and there was 56% less soil NO_3^- available in the ant exclosures

than in the disturbance controls ($F_{1,4} = 13.37$, $P = 0.02$; Fig 7E).

The effect of ants on both soil NH_4^+ and NO_3^- availability changed from before the

adelgid colonized the plots to afterwards (Fig. 8). We note that the smaller sample size (i.e., one-

half the data) for each analysis reduced statistical power. Prior to adelgid colonization, there was

no effect of ant treatment, canopy treatment, or change through time on NH_4^+ availability ($F_{1,4} =$

0.19, $P = 0.68$; $F_{3,3} = 0.31$, $P = 0.82$, $F_{1,223} = 0.63$, $P = 0.43$, respectively; Fig. 8A, left).

However, after the adelgid colonized the plots, there was a significant canopy × ant treatment

interaction ($F_{3,4} = 11.51$, $P = 0.02$) and a marginal effect of ant treatment alone ($F_{1,4} = 5.62$, $P =$

0.08), but still no effect of time on soil NH_4^+ availability ($F_{1,207} = 0.26$, $P = 0.61$; Fig. 8A, right).

In contrast, prior to adelgid colonization, we detected a marginal effect of ant treatment ($F_{1,4} =$

6.48, $P = 0.06$) and an interaction between ant treatment and canopy treatment ($F_{3,4} = 6.09$, $P =$

0.06) as NO_3^- overall declined slightly through time ($F_{1,223} = 5.34$, $P = 0.02$; Fig. 8B). After the

adelgid colonized the plots, the canopy × ant treatment interaction term and the time effect were

no longer significant ($F_{3,4} = 3.03$, $P = 0.16$; $F_{1,207} = 0.16$, $P = 0.69$), but the effects of ants on

NO_3^- availability were stronger ($F_{1,4} = 9.24$, $P = 0.04$; Fig. 8B).

Path modeling

The path model, for which we were able to use only data for multiple soil ecosystem

variables that were collected prior to adelgid infestation, explained 64% of the variance in the

data (Fig. 9). Canopy composition directly affected ant diversity and abundance, decomposition

rate of cellulose (under the intact hardwood canopy) and lignin (in logged stands), and soil NO_3^-

availability (in girdled stands and under the intact hardwood canopy). After accounting for

canopy effects, ant diversity and abundance were correlated with NH_4^+ availability. After

accounting for both canopy effects and ant effects, decomposition of cellulose and lignin further

affected NO_3^- availability. Indirect effects of logging (through ant abundance and lignin) on NO_3^-

availability were stronger than the direct effects (dotted line in Fig. 9). Because ant assemblage

data were transformed to differences from controls prior to path analysis, path model effects are

complimentary, but not directly comparable to ANCOVA results.

DISCUSSION

The loss of dominant species in forests caused by species invasions and human activities

can have direct impacts on ecosystem processes, but the differences between impacts attributable

to direct changes in forest structure and those attributable to subsequent shifts in associated

faunal assemblages have not been quantified previously. Here, we manipulated forest canopy

cover at hectare scales and simultaneously manipulated abundance of ground-dwelling ants at

meter scales to explore how loss of *Tsuga canadensis* – a foundation species – altered soil

ecosystem variables both directly and indirectly via canopy effects on ant assemblages. The large

spatial and temporal scale of the experiment limited the number of replicate plots (but we note

that from the perspective of a 4-mm long ["medium-sized"] ant a *ca.* 1–ha plot is about the same

size as the five-borough New York City area is to an individual person), but it was still possible

to analyze the design as a split-plot ANCOVA (Ellison et al. 2005b), which we used to identify

differences among treatments that are expected to occur as *T. canadensis* declines throughout

eastern North America.

The ant exclosures successfully reduced ant abundance (Figs. 3, 4; see also Wardle et al.

2011) while having no significant effects on vegetation composition or structure (Fig. 2). Other

invertebrates also were likely excluded by the treatments, but given the large decline in ant

abundance and their known effects on forest soils in northeast North America (Lyford 1963) and

elsewhere (Frouz et al. 2008, Jelikova et al. 2011, Wardle et al. 2011, Del Toro et al. 2012), it

seems reasonable to infer that the differences we observed in soil ecosystem variables between

the exclosure and control subplots can be attributed to changes in ant activity ultimately caused

by the canopy manipulations (Fig. 9). The changes that we observed in ant assemblages (Figs. 3,

5; see also Ellison et al. 2005a, Rohr et al. 2009, Sackett et al. 2011, Ribbons 2014), nitrogen

availability, energy flux, decomposition rates, and plant assemblages (Figs. 2, 6−8; see also

Orwig et al. 2008, 2013) as a function of the canopy manipulations were consistent with previous

observational studies. In addition, we were able for the first time to determine that cellulose

decomposition was affected directly by changes in forest canopy structure (a proxy for associated

environmental changes, such as changes in light availability [Fig. 1] and temperature at the forest

floor), whereas soil nitrate availability was indirectly enhanced by reduction in abundance of

ground-dwelling ants (Fig. 9).

Changes in soil ecosystem variables directly attributable to canopy-level changes most likely reflect changes in microclimate such as temperature and moisture (Lustenhower et al. 2012). Cellulose decomposition was relatively high in hardwood control plots and relatively low in the logged plots, where warmer and drier conditions slow decomposition (see also Donelly et al. 1990). The results of our path model (Fig. 9) further indicate that decomposition of both cellulose and lignin can strongly affect NO_3^- availability, with more rapid decomposition associated with higher availability. Effects of hemlock decline on microclimate and decomposition may also reduce NO_3^- availability independent of any effects of associated changes in ant activity. We note that we measured decomposition only over a relatively short period of time using standard substrates and only three years after canopy manipulations were complete. Effects of *T. canadensis* loss on soil microclimate and the biotic community will continue to change, and possibly increase, over time. Vegetation data from this study also confirmed that experimental removal of *T. canadensis* affects plant community composition (Fig. 2; see also Farnsworth et al. 2012 and Orwig et al. 2013), which might alter plant nitrogen uptake from the soil pool. However, ant exclosures did not alter small-scale plant assemblages independently of the effects of the canopy manipulations (Fig. 2).

Among the soil ecosystem variables that we measured, exclusion of ants reduced only soil NO_3^- availability (see also Del Toro et al., *unpublished data*). Initially, we hypothesized that ant activity would increase soil nitrogen availability directly by increasing the rate of nitrogen recycling via decomposition, especially given that the most commonly observed species are known to nest in rotting wood (Ellison et al. 2012). However, differences in ant abundance between ant exclosures and their associated controls had no significant effect on decomposition rates. An alternative explanation for the effects of ants on NO_3^- availability parallels observations

made by Jelikova and Frouz (2014). They suggested that ants may increase soil N locally

through "microbial priming" (sensu Cheng 1999) when they bring honeydew and other insect

exudates to their nests. In fact, the more common *Formica* species in our girdled and logged

plots do tend aphids and scale insects on seedlings, saplings, and trees (Marquis et al. 2014); this

honeydew could be contributing to increased NO_3^- availability outside of the ant exclosures. We

note further that *Formica* species were not collected in any of the ant manipulation subplots

within the hemlock control plots (see also Ellison et al. 2005a). Soil and litter bioturbation by

ants foraging and building nests may also alter nutrient cycling patterns, aerate the soil, and

increase the movement of soil water (Nkem et al. 2000) – all activities that may increase

microbial activity. We hypothesize that as large-scale loss of *T. canadensis* continues, that

increasing abundance of *Formica* species will lead to increased soil NO_3^- availability. This

process could be important in these transitioning ecosystems as ants may facilitate nutrient

retention when plant biomass (and thus nutrient uptake) is reduced.

Clearly, the loss of foundation species will have direct impacts on ecosystem function,

but indirect effects, such as shifts in species interactions, while less studied, also may shape

ecosystems recovery following disturbance. Our data indicate that changes in ant abundance can

regulate some soil ecosystem variables in forests, but it remains an open question if these

patterns will scale over time to influence the trajectory of ecosystems after the loss of *T.*

canadensis.

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Table 1. Large (>|0.2|) loadings of the first two principal component scores for the understory vegetation sampled along transects in the Harvard Forest Hemlock Removal Experiment.

Species	PC-1	PC-2
Lysimachia borealis (Raf.) U. Manns & A. Anderb.	−0.30	…
Aralia nudicaulis L.	−0.29	…
Dendrolycopodium obscurum (L.) A. Haines	−0.29	…
Maianthemum canadense (Desf.) Greene	−0.28	…
Mitchella repens L.	−0.26	…
Dennstaedtia punctilobula (Michx.) T. Moore	−0.25	…
Huperzia lucidula (Michx.) Trevis.	−0.22	…
Oclemena acuminata (Michx.) Greene	−0.20	…
Rhododendron periclymenoides (Michx.) Shinners	…	−0.35
Ilex verticillata (L.) Gray	…	−0.34
Berberis thunbergii DC.	…	−0.32
Epigaea repens L.	…	−0.32
Lonicera canadensis Bartr. ex. Marsh.	…	−0.32
Lysimachia qauadriflora Sims.	…	−0.32
Osmundastrum cinnamomea (L.) C. Presl.	…	+0.22

Notes: Species are sorted by absolute value of the loadings. Nomenclature follows Haines (2011).

Table 2. Occurrence ("X") between 2006 and 2014 of ant species in the ant exclosure treatments (C: control; D: Disturbance control; X: ant exclosure) within the different canopy treatments (Hemlock: Hemlock control; Girdled: Hemlocks girdled; Logged: Hemlocks cut and removed; Hardwood: Hardwood control).

Ant species	Hemlock			Girdled			Logged			Hardwood		
	C	D	X	C	D	X	C	D	X	C	D	X
Aphaenogaster fulva Roger, 1863					X					X	X	
Aphaenogaster picea (Wheeler, 1908)	X	X	X	X	X	X	X	X	X	X	X	X
Camponotus herculeanus (Linneaus, 1758)							X					
Camponotus nearcticus Emery, 1893						X	X	X			X	
Camponotus novaeboracensis (Fitch, 1855)					X		X	X	X		X	
Camponotus pennsylvanicus (DeGeer, 1773)	X	X	X	X	X	X	X	X	X	X	X	X
Formica argentea Wheeler, 1902										X		
Formica aserva Forel, 1901				X			X			X	X	
Formica incerta Buren, 1944								X				
Formica lasioides Emery, 1893										X		
Formica neogagates Viereck, 1903								X	X	X		
Formica neorufibarbis Emery, 1893								X			X	
Formica pergandei Emery, 1893							X			X		
Formica subaenescens Emery, 1893				X			X				X	
Formica subintegra Wheeler, 1908				X	X		X	X		X	X	X
Formica subsericea Say, 1836							X	X	X	X	X	X
Lasius alienus (Foerster, 1850)	X	X	X	X	X		X			X	X	X
Lasius interjectus Mayr, 1866						X						
Lasius nearcticus Wheeler, 1906												X
Lasius umbratus (Nylander, 1846)	X		X			X			X	X	X	X
Myrmecina americana Emery, 1895				X								
Myrmica detritinodis Wheeler, 1917				X				X	X			
Myrmica nearctica Weber, 1939							X	X	X			
Myrmica punctiventris Roger, 1863				X	X	X	X	X	X	X	X	X
Myrmica sp. AF-scu sensu A. Francoeur							X	X	X			
Myrmica sp. AF-smi sensu A. Francoeur				X	X		X	X	X			
Stenamma brevicorne (Mayr, 1886)		X	X	X								
Stenamma diecki Emery, 1895								X				
Stenamma impar Forel, 1901	X	X	X		X		X		X	X		
Stenamma schmitti Wheeler, 1903			X	X					X	X		
Tapinoma sessile (Say, 1836)					X			X	X			
Temnothorax longispinosus (Roger, 1863)				X	X	X	X					
Total species richness	**5**	**5**	**7**	**13**	**11**	**7**	**14**	**18**	**14**	**15**	**13**	**8**

Note: Nomenclature follows Ellison et al. (2012).

Table 3. Large (>|0.2|) loadings of the first two principal component scores for the ant

assemblages from the ant manipulation subplots in the Harvard Forest Hemlock Removal

Experiment.

Species	PC-1	PC-2
Camponotus pennsylvanicus	0.56	…
Formica neogagates	0.46	…
Formica subintegra	0.49	…
Formica incerta	0.34	…
Myrmica punctiventris	…	0.65
Stenamma diecki	…	0.39
Tapinoma sessile	…	0.38
Myrmica sp. AF-smi	…	0.37
Lasius nearcticus	…	0.28

Note: Species are sorted by absolute value of the loadings.

Figure Legends

Fig. 1. Changes in light availability (expressed as global site factor [GSF]: the weighted average of direct + diffuse radiation relative to full-sun, above-canopy, conditions) in the Harvard Forest Hemlock Removal plots from 2005 – 2014. Each point represents the mean GSF of 25 photographs taken at 15-m intervals across each plot; left and right columns of panels represent, respectively, photographs taken in the summer (deciduous trees in full leaf) or winter (deciduous trees leafless) in the four canopy-manipulation treatments (top to bottom rows, HC: Hemlock control; G: Girdled; L: Logged; HD: Hardwood control). Different symbols are used for plots in the ridge (solid circle) and valley (solid triangle) blocks. The vertical dashed line indicates the year (2009) when we first detected the adelgid in the plots. Regression lines (with shaded 95% confidence intervals; data pooled for both blocks) were fit to those portions of data identified as being different using piecewise linear regression in the segmented package of the R statistical software system.

Fig. 2. Vegetation composition through time expressed as principal component scores in the canopy-manipulation plots (light gray to dark gray filled circles; larger circles are 2003 [lightest gray] and 2014 [darkest gray]) and in the ant-exclosure subplots (filled squares: magenta: control; tan: disturbance control; blue: exclosures). PC scores have been square-root transformed to provide more visual separation among the points. See Table 1 for loadings on each of these principal axes. Abbreviations for canopy-manipulation treatments as in Fig. 1.

Fig. 3. Effects of ant and canopy manipulations on ant species composition the Harvard

Forest Hemlock Removal Experiment. (A) Temporal changes in mean abundance of eight ant genera in each of the three ant-manipulation × four canopy-manipulation treatments. The black line represents total abundance: the sum of abundances of individual genera. (B) Temporal changes in effective number of species in each of the three ant manipulations (different line types) × four canopy treatments (colors). Abbreviations for canopy-manipulation treatments as in Fig. 1.

Fig. 4. ANOVA (interaction) plots showing means (± 1 SE) of total ant abundance (A, C) and effective number of species (B, D) for each combination of ant × canopy treatment. Interaction plots are illustrated both for the entire time-course of the experiment (A, B) and for pre- and post-adelgid conditions (C, D). The lines connecting the symbols pass through the mean of the subplots in the canopy-manipulation plots. The vertical lines illustrate ± 1 SE of the means of the subplots in the canopy-manipulation plots in each of the blocks. Abbreviations for canopy-manipulation treatments as in Fig. 1; colors indicate ant-manipulation treatments as in Fig. 2.

Fig. 5. Ant assemblage composition through time in each of the three ant-manipulation × four canopy-manipulation treatments in the valley (black) and ridge (red) blocks. Trajectories end in 2014 at the points of the arrows. See Table 3 for loadings on each of these principal axes. Abbreviations for canopy-manipulation treatments as in Fig. 1.

Fig. 6. Time-series plots of differences among soil ecosystem variables as a function of canopy and ant manipulation treatments on cellulose decomposition (A), lignin decomposition (B), soil respiration (C), ammonium (NH_4^+) availability (D) and nitrate (NO_3^-) availability (E).
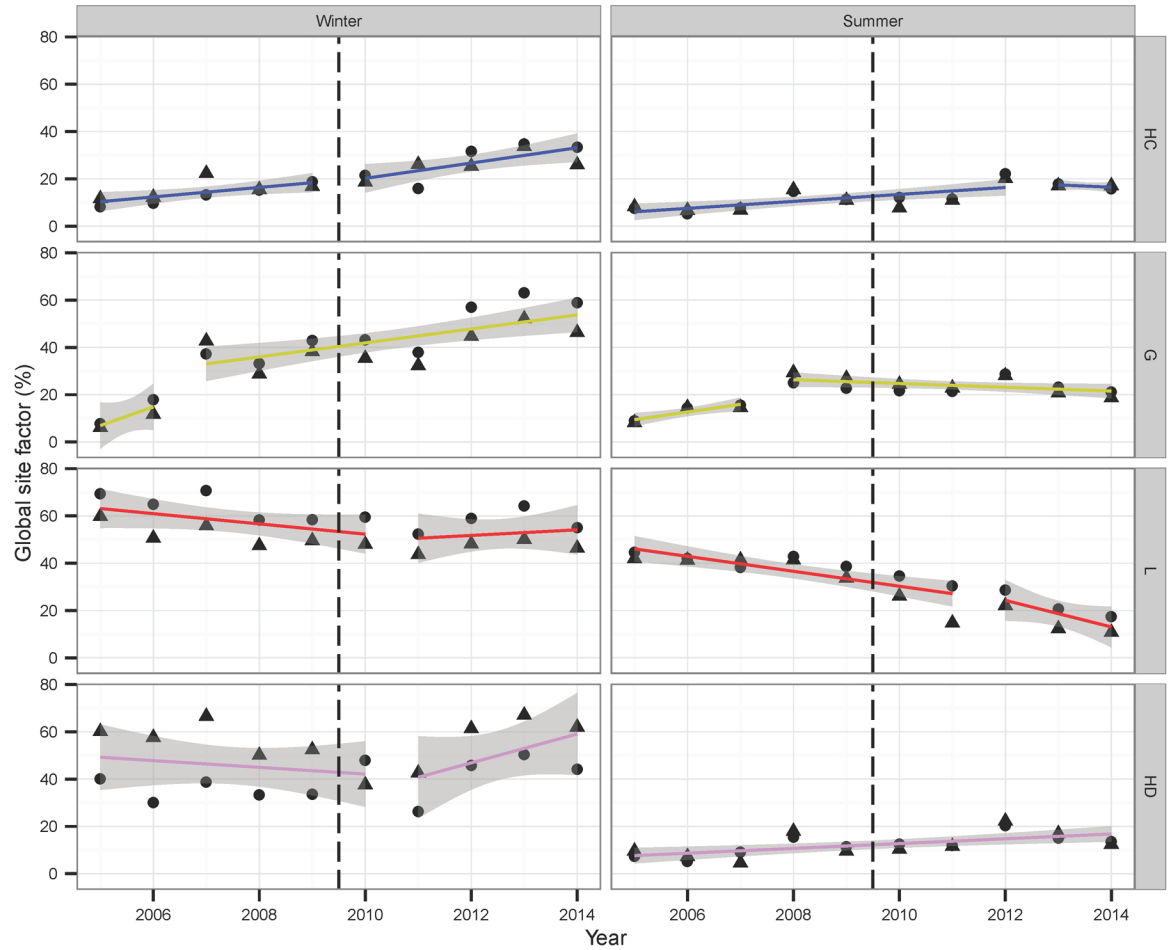
Colors and line types as in Fig. 3B. Note that data for soil respiration, NH_4^+ availability, and NO_3^- availability are presented as differences from ant manipulation controls. Abbreviations for canopy-manipulation treatments as in Fig. 1.
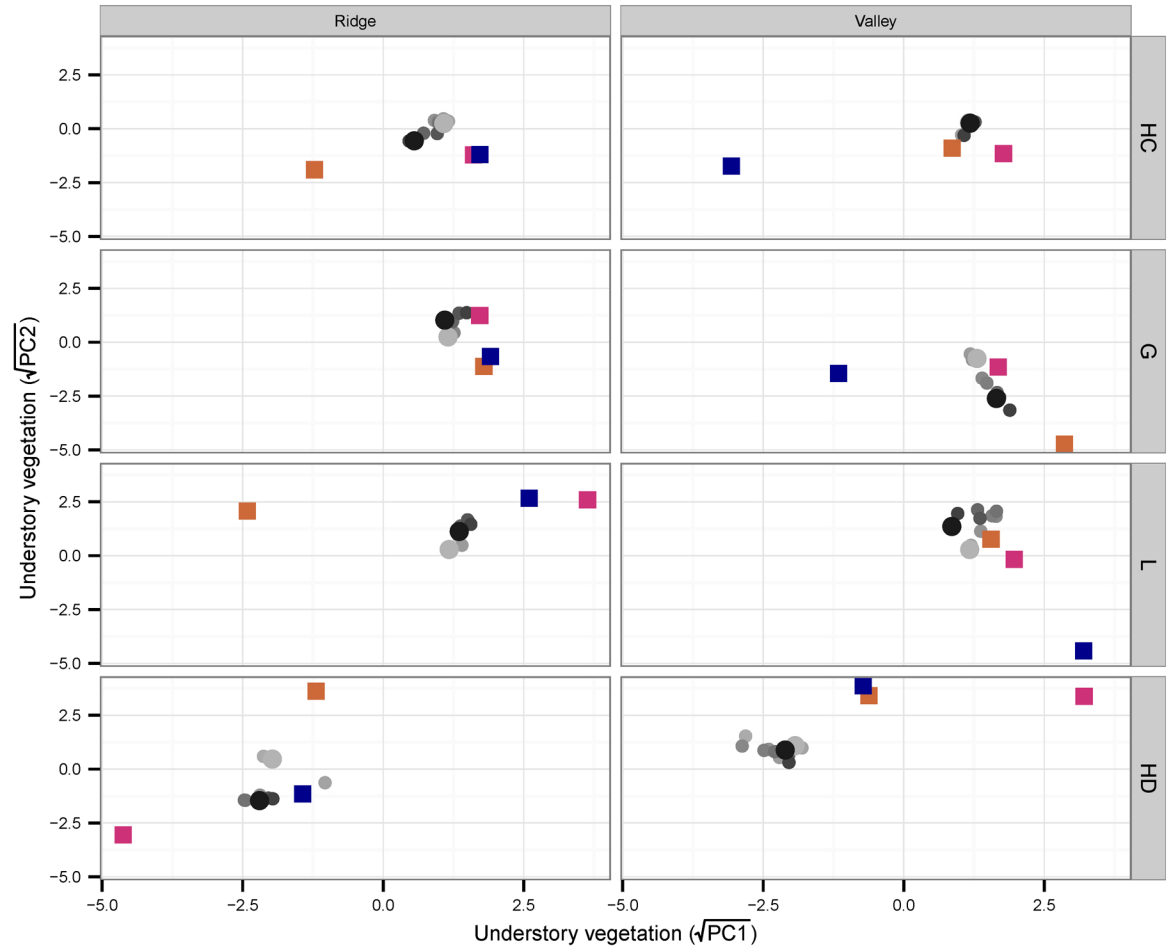
Fig. 7. ANOVA (interaction) plots of the effects of canopy and ant manipulation treatments on cellulose decomposition (A), lignin decomposition (B), soil respiration (C), ammonium (NH_4^+) availability (D) and nitrate (NO_3^-) availability (E). Symbols and lines as in Figure 4. These analyses were done over the entire time-course of the available data. As in Fig. 6, data shown are differences from ant manipulation controls for soil respiration, NH_4^+ availability, and NO_3^- availability. Abbreviations for canopy-manipulation treatments as in Fig. 1; colors indicate ant-manipulation treatments as in Fig. 2.

Fig. 8. ANOVA (interaction) plots of the effects of canopy and ant manipulation treatments on ammonium (NH_4^+) availability (A) and nitrate (NO_3^-) availability (B) before and after the adelgid colonized the canopy-manipulation plots. These analyses were done on differences from ant manipulation controls. Abbreviations for canopy-manipulation treatments as in Fig. 1; colors indicate ant-manipulation treatments as in Fig. 2; lines as in Fig. 4.
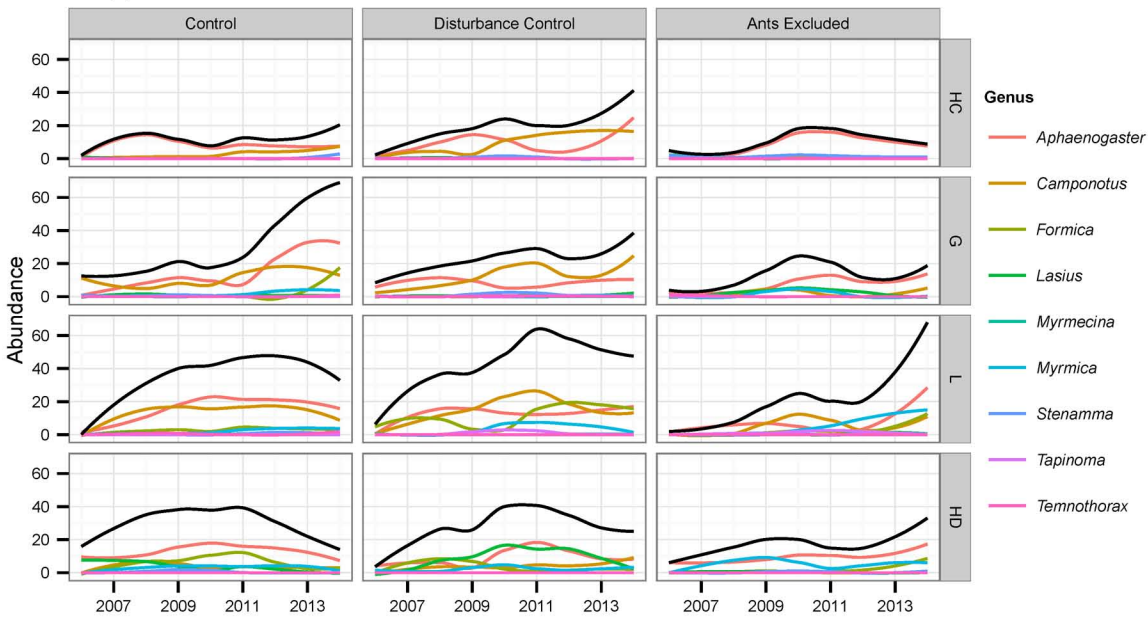
Fig. 9. Direct and indirect effects of canopy structure and ant assemblages on soil ecosystem variables prior to adelgid colonization of the plots examined with partial least squares path modeling. Arrows represent all hypothesized relationships. Relationships with a direct or indirect effect >0.3 are labeled and colored; blue arrows indicate positive relationships whereas red arrows indicate negative relationships (all relative to hemlock controls). The dashed line

indicates that the indirect effect of logging on NO_3^- is greater than its direct effect. Shading of

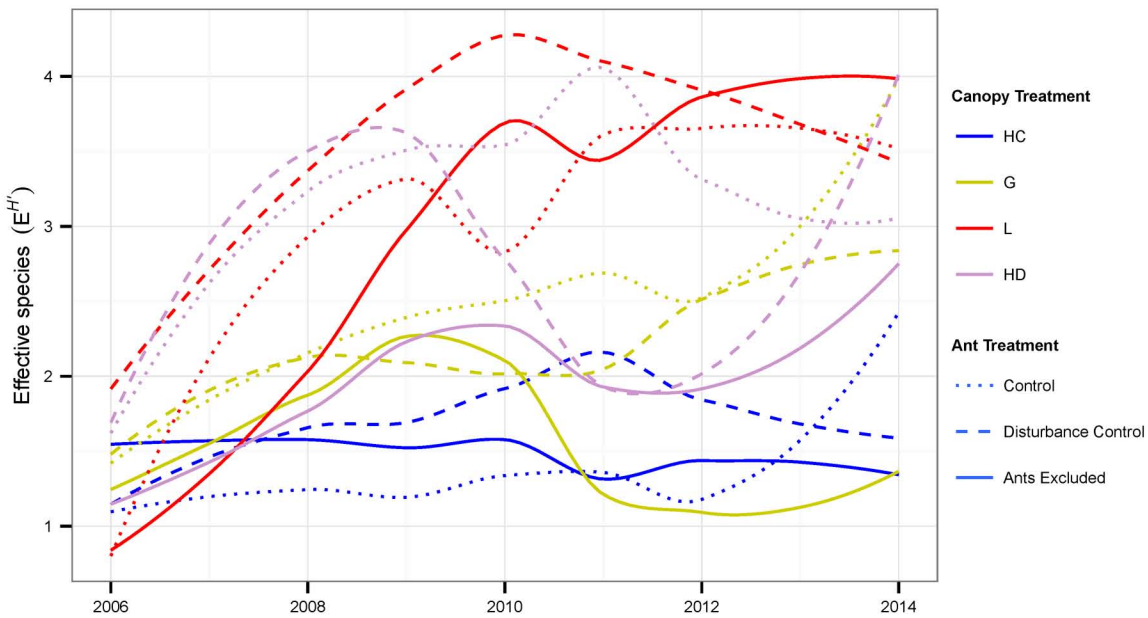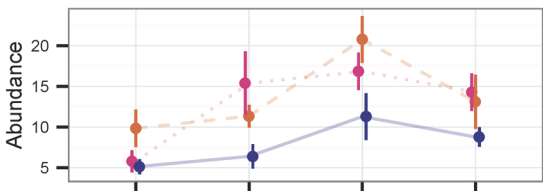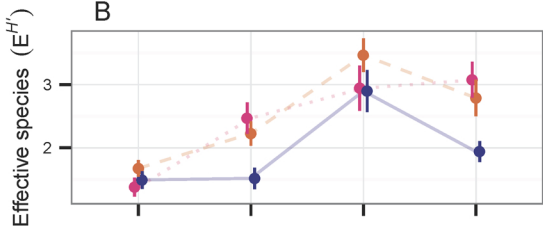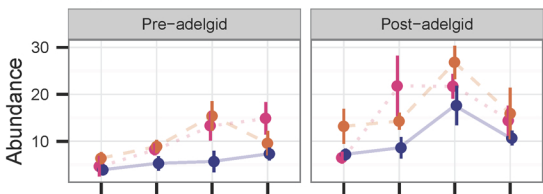Canopy Treatment and Decomposition sections is for clarity only.
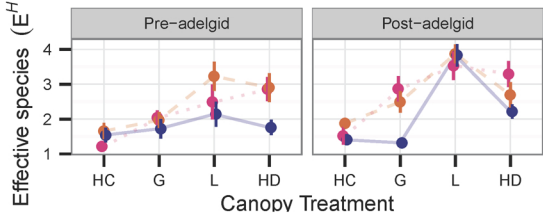
A

B

C

C

Pre-adelgid

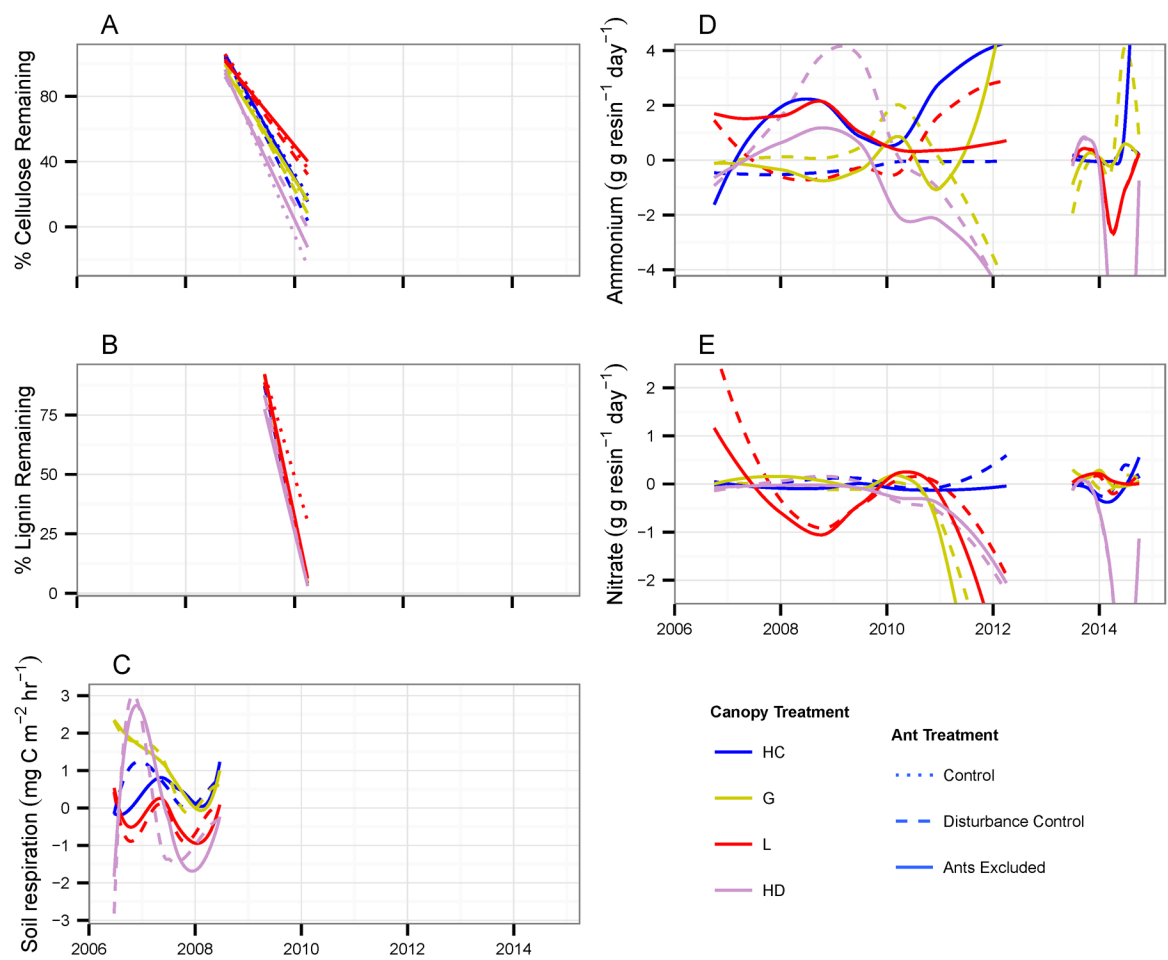Post-adelgid

Pre-adelgid

Post-adelgid

Canopy Treatment

HC   G   L   HD

Ant Treatment

● Control
● Disturbance Control
● Ants Excluded

Figure panels A–E.

A — % Cellulose Remaining vs. year (2006–2014)

B — % Lignin Remaining vs. year (2006–2014)

C — Soil respiration (mg C m^{-2} hr^{-1}) vs. year (2006–2014)

D — Ammonium (g g resin^{-1} day^{-1}) vs. year (2006–2014)

E — Nitrate (g g resin^{-1} day^{-1}) vs. year (2006–2014)

Canopy Treatment
- HC
- G
- L
- HD

Ant Treatment
- Control
- Disturbance Control
- Ants Excluded

Ant Treatment

- Control
- Disturbance Control
- Ants Excluded

A

B

Ant Treatment   Disturbance Control   Ants Excluded