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Research paper

# Invasive insect effects on nitrogen cycling and host physiology are not tightly linked

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Invasive insects may dramatically alter resource cycling and productivity in forest ecosystems. Yet, although responses of individual trees should both reflect and affect ecosystem-scale responses, relationships between physiological- and ecosystem-scale responses to invasive insects have not been extensively studied. To address this issue, we examined changes in soil nitrogen (N) cycling, N uptake and allocation, and needle biochemistry and physiology in eastern hemlock (*Tsuga canadensis* (L) Carr) saplings, associated with infestation by the hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand), an invasive insect causing widespread decline of eastern hemlock in the eastern USA. Compared with uninfested saplings, infested saplings had soils that exhibited faster nitrification rates, and more needle $^{15}N$ uptake, N and total protein concentrations. However, these variables did not clearly covary. Further, within infested saplings, needle N concentration did not vary with HWA density. Light-saturated net photosynthetic rates ($A_{sat}$) declined by 42% as HWA density increased from 0 to 3 adelgids per needle, but did not vary with needle N concentration. Rather, $A_{sat}$ varied with stomatal conductance, which was highest at the lowest HWA density and accounted for 79% of the variation in $A_{sat}$. Photosynthetic light response did not differ among HWA densities. Our results suggest that the effects of HWA infestation on soil N pools and fluxes, $^{15}N$ uptake, needle N and protein concentrations, and needle physiology may not be tightly coupled under at least some conditions. This pattern may reflect direct effects of the HWA on N uptake by host trees, as well as effects of other scale-dependent factors, such as tree hydrology, affected by HWA activity.

***Keywords***: *Adelges tsugae*, eastern hemlock, hemlock woolly adelgid, invasive species, N allocation, N cycling, photosynthesis, *Tsuga canadensis*.

## Introduction

Invasive insects may dramatically alter tree growth and survival, with cascading effects on forest ecosystems. In addition to altering forest composition, tree declines may lead to changes in resource cycling and other ecosystem functions (Schowalter et al. 1986, Gandhi and Herms 2010, Nuñez et al. 2010, Hicke et al. 2012). However, although ecosystem-scale responses and responses of individual trees are linked, little is known about feedback between changes in ecosystem processes and responses of individual trees (Pinkard et al. 2011). For example, while the hemlock woolly adelgid (HWA; *Adelges tsugae* Annand), an invasive aphid-like insect, has been shown to alter physiology and morphology in eastern hemlock (*Tsuga canadensis* (L.) Carr) (Broeckling and Salom 2003, Gonda-King et al. 2012, Domec et al. 2013, McDonald 2013), as well as alter soil nitrogen (N) cycling in infested forests (Jenkins et al. 1999, Kizlinski et al. 2002, Yorks et al. 2003, Orwig et al. 2008), few studies have examined whether changes in soil cycling may lead to changes in N uptake or needle physiology. Because of the devastating effects invasive insects may have on host health and

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mortality, more research on links between responses at the tree and ecosystem levels are needed to understand the mechanisms that regulate forest responses to these insects (Boyd et al. 2013).

Invasive insects undergo large population increases at some point after introduction, leading to extensive defoliation (Liebhold et al. 1995, Davidson et al. 1999, Anagnostakis 2001). As a result, invasive insects may lead to substantial biochemical and physiological responses by trees, such as changes in N and carbohydrate allocation (Pinkard et al. 2011). These changes may exacerbate the effects of invasive insects on tree health, as such changes may accelerate death through carbon starvation (McDowell and Sevanto 2010, Sala et al. 2010, Domec et al. 2013). Alternatively, the extensive defoliation of host trees may indicate a lack of effective defenses (Sakai et al. 2001) and limited physiological responses. For example, HWA infestation generally leads to extensive defoliation and mortality of eastern hemlock (McClure 1991*b*, Royle and Lathrop 1997, Orwig and Foster 1998), suggesting eastern hemlock has no natural defenses to the HWA (McClure 1995, Montgomery et al. 2009, Vose et al. 2013). Rather, average minimum winter temperature (Morin et al. 2009, Orwig et al. 2012, Eschtruth et al. 2013), summer drought (Eschtruth et al. 2013) and foliar nutrient concentrations (McClure 1991*a*, Pontius et al. 2006, Ingwell et al. 2009, Eschtruth et al. 2013) may play key roles in regulating tree mortality rates.

A potential indirect effect of eastern hemlock defoliation is increased N uptake by healthy trees (Block et al. 2012). Defoliation may increase soil N availability by reducing N uptake by infested trees (Orwig et al. 2008, Block et al. 2012). Also, HWA infestation may lead to increased soil N mineralization rates (Jenkins et al. 1999, Kizlinski et al. 2002, Yorks et al. 2003, Orwig et al. 2008), due to changes in litter quality and litterfall (Stadler et al. 2005, 2006), and rates of litter decomposition (Cobb et al. 2006). Faster N mineralization can lead to increased soil mineral N concentrations (van Cleve et al. 1990, van Breemen et al. 1998), increased N uptake by plants and increased leaf N concentrations (Kellomäki and Wang 1997, Rygiewicz et al. 2000, Hobbie and Gough 2002, Lewis et al. 2004). Because of the central role N plays in photosynthesis, light-saturated net photosynthetic rates ($A_{sat}$) generally increase with increasing leaf N concentrations (Field and Mooney 1986, Evans 1989), suggesting $A_{sat}$ may increase if soil N increases (Norby et al. 1999). However, research suggests the HWA may negatively affect $A_{sat}$ in eastern hemlock (McDonald 2013, Gonda-King et al. 2014), as the HWA is associated with reduced stomatal conductance ($g_s$) and other changes in plant–water relations (Gómez et al. 2012, Gonda-King et al. 2012, Domec et al. 2013).

Accordingly, the HWA may affect eastern hemlock both by altering N uptake by surviving trees and by inducing changes in N allocation within infested trees. However, other host responses to the HWA (e.g., reductions in $g_s$) may mitigate physiological responses to changes in soil N cycling and N uptake. To address this issue, we conducted a study that addressed four questions: (i) does HWA infestation affect N uptake and allocation in eastern hemlock through effects on soil N pools and fluxes; (ii) does HWA infestation affect needle biochemistry and physiology; (iii) do HWA effects on needle biochemistry and physiology vary with adelgid density within a tree; and (iv) do biochemical and physiological responses to the HWA reflect changes in N allocation to needles? We addressed these questions using two complementary experiments. In Experiment 1, we examined changes in soil N pools and fluxes associated with HWA infestation, and compared N uptake and allocation patterns in infested and uninfested eastern hemlock saplings. In Experiment 2, we examined the role of differences in HWA density within saplings on needle biochemistry and physiology, and whether changes in these variables reflected changes in N allocation to needles.

## Materials and methods

### *Experiment 1 (Soil N cycling; N uptake and allocation)*

***Study site*** Experiment 1 was conducted in the Black Rock Brook watershed in Black Rock Forest, Orange County, NY, USA (41.01°N, 74.01°W). Black Rock Forest is a 1530 ha natural area within the New York–New Jersey Highlands Physiographic Province, which overlies Precambrian gneiss, schists and limestones (Schuberth 1968). Black Rock Brook is a north-facing watershed encompassing ~100 ha. Red oak (*Quercus rubra* L.), chestnut oak (*Quercus prinus* L.) and eastern hemlock dominate tree composition in the watershed (Mates-Muchin 2006). Prior to invasion by the HWA, eastern hemlock was the dominant tree species in ~38 ha of the watershed, as estimated by aerial photography and GIS (W. Schuster, personal communication). The remainder of the watershed generally is dominated by oak. Elevation ranges from 160 to 340 m above sea level (a.s.l.). Mean annual precipitation is ~120 mm, while mean monthly air temperatures range from −2.7 °C in January to 23.4 °C in July (Schuster et al. 2008).

Three comparatively uninfested and three infested hemlock-dominated 40 × 40 m plots were randomly located in hemlock-dominated areas within the watershed. Hemlock dominance in each plot was corroborated using an index of hemlock relative importance, which was based on the average of hemlock basal area and density per plot in relation to all of the trees in the plot. In all plots, all trees >1 cm diameter at breast height (DBH) were identified following Brockman (1986) and measured for DBH. These data were then used to calculate basal area and density of each tree species. Based on relative basal area and frequency, hemlock-dominated plots contained ~70% eastern hemlock on average and ~20% oak on average.

### *Sapling selection*

Crown-level defoliation of all hemlock trees within each plot was visually scored in one of five categories: 0–5, 5–25, 25–50,

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50–75 and 75–100% defoliated (Orwig and Foster 1998). Trees that were completely defoliated were considered dead. Based on this approach, infested saplings had extensive crown defoliation (>50% on average), while uninfested saplings exhibited limited crown defoliation (<25% on average). Mean crown defoliation within each infested-hemlock plot was >50% and plot-level hemlock mortality was >50% during the study period, while uninfested-hemlock plots exhibited limited defoliation and mortality of hemlock (<25% defoliation and <10% mortality, on average). In each plot, four hemlock saplings ~5 m tall were randomly selected from the corresponding infestation category for that plot for a total of 24 saplings sampled (two infestation categories × three plots per category × four saplings per plot).

### Soil N characteristics

Soil N characteristics were assessed in June 2005 at each of the study saplings in each plot. Net ammonification and nitrification rates were measured using field incubation methods modified from Robertson et al. (1999). Two PVC pipes (20 × 6.35 cm (diameter)) were inserted 10 cm into soil ~1 m east of each study sapling. One pipe (initial core) was removed immediately with soil intact, while the second pipe (final core) remained inserted for 28 days before being removed. The final core was fitted with a cap to minimize moisture changes and to prevent N leaching by precipitation and had a 0.5-cm hole drilled through the side for ventilation.

After collection, soil samples were sieved through a 4 mm screen to remove rocks and large roots. Two subsamples were weighed per sample; a 4 g (±0.05 g) sample for $NH_4^+$ and $NO_3^-$ extraction, and a 4 g sample for gravimetric water content (dried at 70 °C to a constant mass; Brady and Weil 2001). Inorganic N was extracted using 20 ml of 2 M KCl. After agitating the soil and KCl mixture at 300 rpm for 1 h, the extract was vacuum filtered using a 7.6 cm (diameter) cellulose fiber filter (#40, Whatman Inc., Piscataway, NJ, USA). Extracts were stored frozen (–20 °C) until analysis. Samples were analyzed for extractable N using a TrAAcs 800 auto-analyzer (Bran+Luebbe, SPX, Charlotte, NC, USA). Ammonium was determined using Berthelot reaction chemistry (Searle 1984), and $NO_3^-$ was determined using hydrazine sulfate (Kamphake et al. 1967). Results were converted to μg N g soil$^{-1}$ based on the volume of KCl used for extraction and the dry mass of the samples. Net ammonification was calculated as the difference between the $NH_4^+$ in the final and initial samples. Net nitrification was calculated as the difference between the $NO_3^-$ in the final and initial samples. Net N mineralization was calculated as the difference between the sum of $NH_4^+$ plus $NO_3^-$ in the final and initial samples.

### $^{15}N$ uptake in situ, N uptake bioassays, and needle N, chlorophyll and protein determination

To compare N uptake by infested and uninfested hemlocks during the growing season, $^{15}N$ was applied on June 28th to the soil surrounding each study sapling. At four locations within 10 cm of each study sapling, 25 ml of deionized water containing 4.5 mg of $^{15}N$ as equal parts (mol:mol) $Na^{15}NO_3$ and $^{15}NH_4Cl$ in $diH_2O$ (98 At% $^{15}N$ in both cases) was injected into the soil at 5 cm depth using a syringe, for a total of 18 mg of $^{15}N$ in 100 ml deionized water applied to each sapling (after Nadelhoffer et al. 1999). The solution was applied in conjunction with the collection of the initial core for soil N analyses. After 28 days, six first-year needles (needles from the 2005 cohort) and six second-year needles (needles from the 2004 cohort) were randomly collected from the crown of each sapling for $^{15}N$ analysis, in conjunction with the collection of the final core for soil N analyses. Needle $^{15}N$ and N concentrations were measured on dried, ground tissue using a mass spectrometer at the Stable Isotope Research Unit, Department of Crop and Soil Sciences, at Oregon State University (Corvallis, OR, USA).

In conjunction with the above procedures, first- and second-year needles were collected for chlorophyll and protein analyses. From each sapling crown, 6–10 needles in each cohort were randomly selected for chlorophyll analysis and six needles in each cohort were randomly selected for protein analysis. Chlorophyll concentration was determined colorimetrically following acetone extraction of fresh tissue (Porra et al. 1989). Protein concentration was determined colorimetrically using a Bio-Rad Protein Assay kit, with bovine serum albumin as the standard (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Nutrient uptake by roots was measured using laboratory bioassays, where the instantaneous influx of tracer-labeled nutrient into excised root is inversely related to the nutrient available to the root in situ. Nutrient uptake bioassays were performed by extracting root samples from fine root bundles collected from the top 15 cm of soil within 1 m of mature hemlock trees. Samples were taken from the O and A horizons and only from the top 10 cm of soil. Excised roots were kept moist and stored at 4 °C until the following day. Roots were then washed with tap water to remove soil, immersed in a presoak solution to remove any loosely bound nutrient, then subjected to a nutrient solution enriched with $^{15}N$-labeled $NH_4^+$ ($NH_4Cl$) to determine N uptake (Jones et al. 1991). Root $^{15}N$ and N concentration measurements were performed as above for needle measurements.

### Experiment 2 (Needle-level biochemistry and physiology)

***Study site*** Experiment 2 was conducted at the Louis Calder Center in Armonk, NY, USA (41.13 °N, 73.73 °W). The Calder Center is a 45 ha natural area operated by Fordham University as a research station within the New England Uplands physiographic region, which overlies Precambrian gneiss, schists and limestones (Schuberth 1968). Red oak dominates tree composition in this forest, while eastern hemlock occurs as scattered trees and in small stands (<1 ha) (J.D. Lewis, unpublished data). Elevation ranges from 140 to 200 m a.s.l. Mean annual

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precipitation and monthly temperatures are similar to conditions at Black Rock Forest.

### Gas exchange measurements

Gas exchange measurements were made on seven randomly selected eastern hemlock saplings during a 3-week period in July 2002. Saplings were similar in size to those used in Experiment 1 (i.e., ~5 m tall). However, HWA infestation was scored based on adelgid density rather than crown defoliation. On each sapling, branchlets were selected to represent Low (<0.5 adelgids per needle on average), Medium (0.5–1.5 adelgids per needle) or High (>1.5 adelgids per needle) adelgid densities. All measurements were made on intact fully expanded, unshaded needles from the most recent fully expanded cohort. Needles were arranged in the cuvette such that self-shading was minimized. After measurement, all branchlets were collected to measure total leaf area and confirm adelgid densities. Needle surface area was estimated using needle length and width. Adelgid densities were assessed by counting, using a stereomicroscope, the number of adelgids at the base of all needles. Measured adelgid densities were (mean ± 1 SE) 0.10 ± 0.03 (Low), 1.33 ± 0.05 (Medium) and 2.32 ± 0.15 adelgids/needle (High). Measured adelgid densities were used in the analyses of adelgid effects on gas exchange variables.

Photosynthesis was measured using an infrared gas analyzer built into a leaf cuvette in an open-flow gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA). All measurements were made using a red:blue light source (LI-6400-02B). The airstream entering the cuvette was maintained at 36.5 Pa $CO_2$ using the LI-6400 computer-controlled $CO_2$ mixing system. Needle temperature was maintained at ~26 °C using thermoelectric coolers mounted on the cuvette. Needle and cuvette air temperatures generally were similar during measurements. Leaf-to-air vapor pressure deficit was generally between 1.2 and 1.8 kPa. Air temperature and leaf-to-air vapor pressure deficit reflected typical ambient conditions during the measurement period.

Photosynthesis was measured at 12 photosynthetic photon fluxes ($Q$) between 20 and 1500 μmol $m^{-2}$ $s^{-1}$ to generate photosynthetic light response ($AQ$) curves. Light response curves were run from high to low $Q$. Measurements were made once needles equilibrated at the target $Q$. Needles were considered equilibrated once conductance ($g_s$) had reached maximum values and the total coefficient of variation for gas exchange parameters was <1% for 1 min. In general, the equilibration period lasted ~5 min. Measurements were initiated at ~09:00 Eastern Standard Time, and typically were completed by 12:00 Eastern Standard Time.

Light response curves were individually analyzed using the non-rectangular hyperbolic model (Prioul and Chartier 1977, Leverenz and Jarvis 1979, Marshall and Biscoe 1980):

$$\Theta A^2 - A(\varphi Q + A_{sat}) + \varphi A_{sat} = 0,$$

where $\Theta$ is the convexity of the light response curve, $A$ is the net photosynthetic rate, $\varphi$ is the maximum apparent quantum yield (the initial slope of the curve), $Q$ is the incident photosynthetic photon flux and $A_{sat}$ is the light-saturated net photosynthetic rate. Curves were fit following Lewis et al. (2000) using the non-linear regression model function in SYSTAT (SPSS, Inc., Evanston, IL, USA). To obtain a correct intercept on the $y$-axis (Shapiro et al. 2004), $\varphi$ and the non-photorespiratory respiration rate in the light ($R_L$) were calculated by linear regression analysis using data collected at $Q$ between 20 and 100 μmol $m^{-2}$ $s^{-1}$. For each curve, the light compensation point ($Q_{comp}$) was calculated as the $Q$ at which $A$ equaled zero.

### Needle N and chlorophyll determination

All needles were harvested immediately following gas exchange measurements. From each sample of needles used for gas exchange measurements, a subsample of needles was randomly selected for chlorophyll and N concentration analysis. Needle chlorophyll concentrations were measured as in Experiment 1. The remaining tissue was dried for 48 h at 60 °C and ground for N concentration analysis. Needle N concentration was measured using a PerkinElmer 2400 Series II CHNS/O analyzer (PerkinElmer, Norwalk, CT, USA).

### Statistical analyses

For Experiment 1, differences in soil N cycling beneath infested and uninfested hemlock saplings were examined using single-factor analysis of variance (ANOVA) with infestation status as the main effect. The effects of infestation status on $^{15}N$ uptake and needle N, chlorophyll and protein concentrations on first- and second-year needles were examined using single-factor repeated-measures ANOVA with infestation status as the between-subject effect and needle cohort as the within-subject effect. Needle cohort was treated as a within-subject effect because measurements of first- and second-year needles on a given sapling were not independent. For Experiment 2, the effects of adelgid density on needle N and chlorophyll concentrations, and variables calculated from $AQ$ curves were examined using linear mixed models, with sapling as a random effect. For both experiments, data were transformed when necessary (ammonification rate, nitrification rate, N mineralization rate, extractable $NH_4^+$, extractable $NO_3^-$, total extractable N and $g_s$) using a $\log_{10}$ transformation to achieve normality and homogeneity of variances. To examine whether soil and needle N properties covaried, correlation analyses were conducted using soil N pools and fluxes versus needle N, protein and chlorophyll concentrations. Regression analyses were conducted to examine whether variation in $A_{sat}$ was associated with variation in $g_s$ and needle N, protein and chlorophyll concentration. For all analyses, treatment effects were considered significant at $\alpha \leq 0.10$, to take into account variability of environmental factors in the field. All analyses except for the linear mixed models were conducted

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using the GLM function in SYSTAT v.9 (SPSS Inc., Chicago, IL, USA). Linear mixed models were run using R package *lme4* in R version 3.1.2 (R Development Core Team 2013).

## Results

### *Experiment 1 (N uptake and allocation in infested and uninfested saplings)*

Soil nitrification was nearly 12-fold greater in soils beneath infested compared with uninfested saplings (Table 1). No other measured soil characteristics clearly differed between infested and uninfested stands. Root $^{15}N$ uptake assays indicated that there was no clear difference in N uptake rates in roots of infested compared with uninfested saplings ($P = 0.267$; data not shown; higher uptake in root assays indicates greater demand for N). Similarly, root N concentration did not clearly differ between infested and uninfested saplings ($P = 0.385$; data not shown).

Needles on infested saplings accumulated significantly more $^{15}N$ than needles on uninfested saplings ($P = 0.007$; Figure 1). Mean needle N ($P = 0.048$) and protein ($P = 0.024$) concentrations were 7 and 31% greater, respectively, in infested compared with uninfested saplings (Figure 1). Needle N concentration was higher in first-year compared with second-year needles (Figure 1; $P < 0.001$), while needle $^{15}N$ and protein concentration did not clearly differ between first- and second-year needles ($P > 0.211$ in both cases). Needle chlorophyll concentrations did not clearly differ between infested and uninfested saplings or between first- and second-year needles ($P > 0.808$ in both cases). The effects of infestation status on all measured variables did not clearly differ between first- and second-year needles ($P > 0.354$ in all cases). No significant correlations were observed between soil N pools and fluxes and needle N, protein and chlorophyll concentrations ($P > 0.178$ in all cases; data not shown).

### *Experiment 2 (Needle-level biochemistry and physiology)*

$A_{sat}$ declined with increasing adelgid density (Figure 2; $\chi^2 = 7.34$, $P = 0.007$). The other variables derived from the *AQ* curves (Table 2), specifically apparent quantum yield, the photosynthetic light compensation point, $R_L$, and the convexity of the *AQ* curve, did not clearly differ with adelgid density ($P > 0.104$ in all cases). As with $A_{sat}$, stomatal conductance ($g_s$) decreased with increasing adelgid density (Figure 2; $\chi^2 = 5.14$, $P = 0.023$). Further, $A_{sat}$ decreased with decreasing $g_s$ (Figure 3). However, $A_{sat}$ did not clearly vary with needle N concentration ($P = 0.572$; data not shown), and $g_s$ did not clearly vary with the needle-to-air vapor pressure deficit ($P = 0.439$; data not shown). Needle chlorophyll

Table 1. Soil N characteristics beneath eastern hemlock saplings either infested or relatively uninfested by the hemlock woolly adelgid (HWA). Data are presented as means ± 1 SE, $n = 12$. *P* values in bold indicate values that are significant at $P \leq 0.10$.

| Soil characteristic | HWA infestation status | | *P* |
|---|---|---|---|
| | Infested | Uninfested | |
| Ammonification rate ($\mu g\ g^{-1}\ day^{-1}$) | 4.37 ± 1.67 | 2.16 ± 0.61 | 0.23 |
| Nitrification rate ($\mu g\ g^{-1}\ day^{-1}$) | 0.154 ± 0.049 | 0.013 ± 0.013 | **0.01** |
| N mineralization rate ($\mu g\ g^{-1}\ day^{-1}$) | 4.52 ± 1.69 | 2.17 ± 0.62 | 0.21 |
| Extractable $NH_4^+$ ($\mu g\ g^{-1}$) | 32.9 ± 5.1 | 31.2 ± 4.2 | 0.79 |
| Extractable $NO_3^-$ ($\mu g\ g^{-1}$) | 3.13 ± 0.43 | 2.53 ± 0.15 | 0.20 |
| Total extractable N ($\mu g\ g^{-1}$) | 36.1 ± 5.2 | 33.7 ± 4.3 | 0.73 |

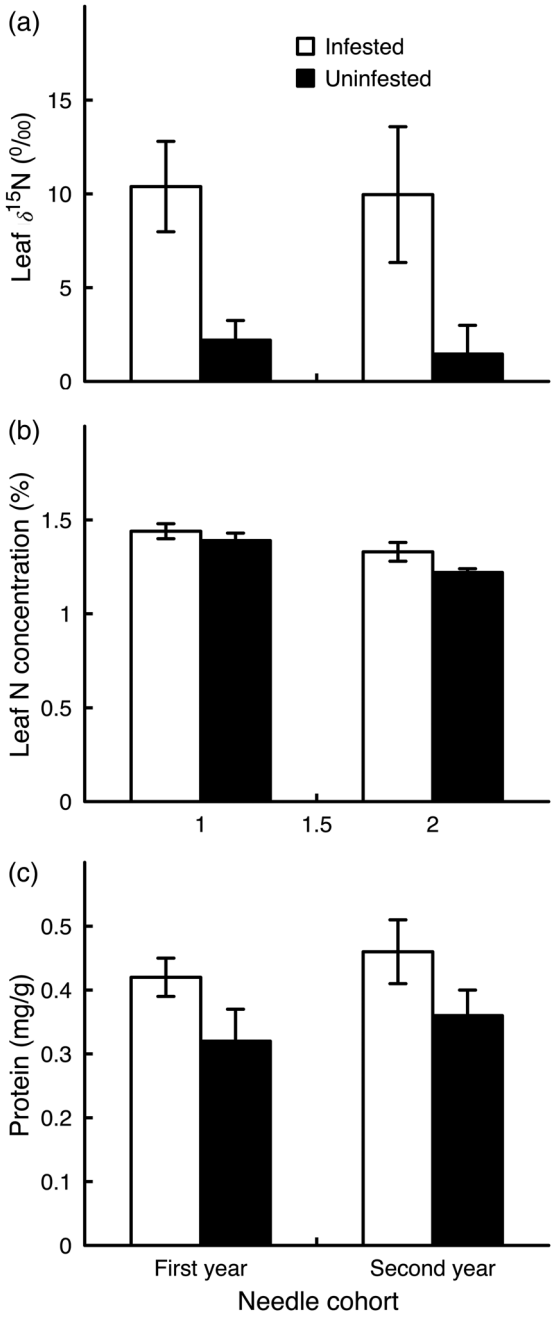


Figure 1. Needle $\delta^{15}N$ 28 days following soil application of equal parts (mol:mol) $Na^{15}NO_3$ and $^{15}NH_4Cl$ (a), total needle N concentration (b) and total needle protein concentration (c) in first- and second-year needles on infested and uninfested hemlock. Values are means ± 1 SE, $n = 12$.

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and N concentrations did not clearly differ among adelgid densities ($P > 0.121$ in both cases). Leaf mass per area also did not clearly vary across adelgid densities (data not shown).

## Discussion

Invasive insects often cause extensive defoliation and mortality of host trees, which may alter N cycling at scales from leaves to landscapes (Orwig 2002, Lovett et al. 2006). Consistent with patterns generally observed in other studies discussed below, HWA infestation was associated with increased soil nitrification rates and needle N concentrations, as well as increased needle protein concentrations and reduced $A_{sat}$. However, although needle N concentrations were higher in infested than uninfested saplings, needle N concentration within infested saplings did not clearly vary with HWA density. Further, the negative effect of the HWA on $A_{sat}$ appeared to be independent of variation in needle N concentration; rather, the reduction was associated with reduced $g_s$. These results suggest that, while HWA infestation may be associated with variation in N cycling from the leaf to the landscape scale, these effects may be decoupled if the HWA alters N uptake and water relations in infested trees. More generally, our results suggest the effects of invasive insects on soil N cycling, tree N dynamics and needle biochemistry and physiology may not be tightly linked, particularly if invasive insects directly affect nitrogen uptake or physiological processes in host trees.

The scale-dependent nature of the relationship between N cycling and HWA infestation may reflect differences in the driving mechanisms at each scale. For instance, at the landscape scale, increased soil nitrification rates have been observed in association with HWA infestation (Jenkins et al. 1999, Stadler et al. 2005, 2006, Orwig et al. 2008). Increased soil N mineralization rates may reflect increased litterfall rates (Stadler et al. 2005, 2006), increased N concentrations in litter (Melillo et al. 1982), or both. At our study site, HWA infestation is associated with faster litterfall rates (Mates-Muchin 2006), and we observed greater needle N concentrations associated with HWA infestation (Figure 1), though needle N concentrations were not

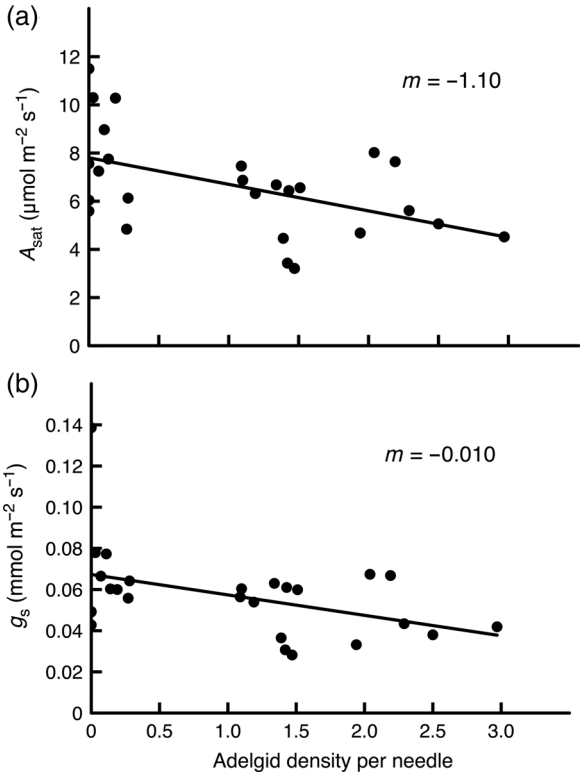


Figure 2. Light-saturated net photosynthetic rates ($A_{sat}$) (a) and stomatal conductance ($g_s$) (b) decreased with adelgid density. The effects of adelgid density on $A_{sat}$ and $g_s$ were tested using a linear mixed model. Accordingly, no coefficient of determination ($r^2$) is shown. $n = 26$.

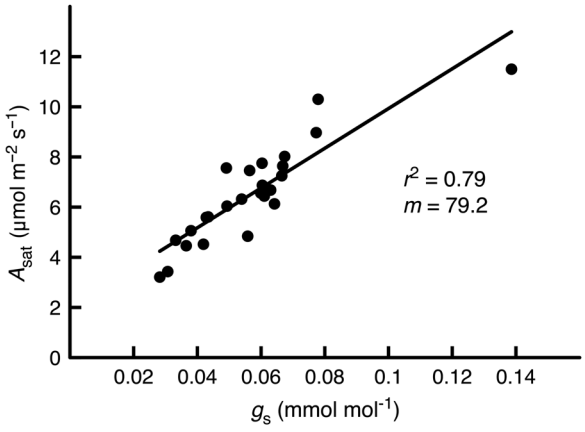


Figure 3. Light-saturated net photosynthetic rates ($A_{sat}$) increased with increasing stomatal conductance ($g_s$) across adelgid densities. The slope ($m$) and coefficient of determination ($r^2$) are shown for the regression.

Table 2. Photosynthetic light response characteristics at Low (<0.5 adelgids per needle on average), Medium (0.5–1.5 adelgids per needle), and High (>1.5 adelgids per needle) adelgid densities. Data are presented as means ± 1 SE. $n = 11$, 9 and 6 for the Low, Medium and High adelgid densities, respectively.

| Variable | Mean adelgid density (adelgid per needle) | | |
|---|---|---|---|
| | Low | Medium | High |
| Convexity (dimensionless) | 0.69 ± 0.06 | 0.76 ± 0.06 | 0.64 ± 0.06 |
| Apparent quantum yield (mol $CO_2$ mol$^{-1}$ photons) | 0.047 ± 0.004 | 0.036 ± 0.004 | 0.040 ± 0.007 |
| Light compensation point (μmol m$^{-2}$ s$^{-1}$) | 7.02 ± 1.00 | 4.35 ± 1.17 | 8.12 ± 1.59 |
| $R_L$ (μmol m$^{-2}$ s$^{-1}$) | 0.37 ± 0.05 | 0.21 ± 0.06 | 0.43 ± 0.12 |



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correlated with soil nitrification rates. Soil nitrification rates may also vary with needle decomposition rates (Cobb et al. 2006). The effect of HWA infestation on decomposition is likely to vary over time, reflecting changes in forest composition and in microclimate, such as changes in soil temperature and moisture associated with canopy defoliation, in addition to changes in needle chemistry (Cobb et al. 2006). Accordingly, it is not clear whether higher soil nitrification rates beneath infested saplings were a result of higher needle N concentrations, faster litterfall rates, increased decomposition rates or other factors.

While soil nitrification rates varied with HWA infestation, changes in soil nitrification rates did not clearly affect needle N concentrations. More generally, we found no clear relationships between needle N concentrations and soil N pools and fluxes. This pattern is consistent with Ollinger et al. (2002), who observed that needle N in uninfested eastern hemlock did not covary with soil N cycling rates, and Block et al. (2012), who observed that soil and leaf N concentrations did not covary in hardwood trees growing in infested eastern hemlock stands. Further, while HWA infestation was associated with a 12-fold increase in soil nitrification rates, the magnitude of this increase was small compared with the sizes of the other N pools and fluxes we measured (Table 1). As a result, differences in soil nitrification rates between infested and uninfested saplings likely did not markedly alter total soil N or the $NO_3^-$ and $NH_4^+$ pools during the study period, consistent with Block et al. (2012), but in contrast to other studies (Jenkins et al. 1999, Orwig et al. 2008). Taken together, our results suggest that HWA effects on needle N concentrations were not tightly linked to HWA effects on soil nitrification rates, and that different mechanisms drove responses at these two scales to HWA infestation.

The lack of clear relationships between needle N concentrations and soil N pools and fluxes suggests HWA infestation may have directly affected needle N concentrations at the tree level (Gómez et al. 2012), as N uptake and needle N concentrations were higher in infested compared with uninfested saplings. Higher needle N concentrations have also been associated with infestation in other studies (McClure 1991*a*, Stadler et al. 2005, Pontius et al. 2006, but see Miller-Pierce et al. 2010). Although the mechanism behind increased uptake is unclear, the pattern suggests the HWA may act as an N sink driving N uptake, may drive hypersensitive or compensatory responses to infestation, or may alter N uptake by releasing factors during feeding. Alternatively, the lack of clear relationships between needle and soil N may reflect increased uptake by surviving trees due to reduced competition from dead trees (Block et al. 2012). The lack of relationships among these variables may also reflect changes in the mycorrhizal community due to hemlock decline (Lewis et al. 2008, Turner et al. 2009), which may affect N uptake, or a lack of N limitation in the study trees (Avolio et al. 2009). There was no clear difference in N demand between infested and uninfested saplings, as root uptake of N was similar between infested and uninfested saplings.

Another possible explanation for the lack of a relationship between needle and soil N is that differences in HWA infestation reflect genetic differences among saplings, which in turn may also affect nutrient uptake. While there is little evidence that there is a genetic basis for differences in HWA susceptibility among eastern hemlock, genetic differences among individuals do influence susceptibility to invasive insects and pathogens in other native trees. Our results may also reflect the confounding effects of one or more physiological or environmental factors that may affect needle N concentration but that were not measured in this study. Accordingly, while our results suggest that HWA infestation directly affected needle N concentrations at the tree level, other factors may also have affected needle N concentrations.

Despite the increased needle N concentrations in infested compared with uninfested saplings, as observed in other studies (McClure 1991*a*, Stadler et al. 2005, Pontius et al. 2006, but see Miller-Pierce et al. 2010), HWA densities within individual saplings did not vary with needle N concentrations. These differences suggest that, while infestation patterns among saplings may be linked to tree-to-tree variation in needle N concentrations, HWA distributions within trees may be independent of needle N concentrations in at least some situations (Gómez et al. 2012). Such a result may occur if HWA population densities were affected by other factors. For example, the lack of a relationship between HWA density and needle N concentration within saplings may reflect stochastic effects of dispersal. In Experiment 2, which examined the relationship between HWA densities and N distribution within saplings, saplings were <25% defoliated, suggesting that these saplings were in the early stages of infestation. During the initial stages of infestation, HWA distributions may reflect the initial sites of infestation within a tree, which reflect the stochastic effects of dispersal rather than N availability. Effects of N availability on HWA distributions within a tree may not be apparent until later stages of infestation, once HWA populations have grown large enough that N is limiting to continued HWA population growth. In addition, Ca, K and P concentrations, which we did not measure, may regulate HWA population densities (Pontius et al. 2006). Regardless, our results suggest that, while infested trees may exhibit increased needle N concentrations compared with uninfested trees, infested and uninfested branches within trees may not exhibit differences in needle N concentrations.

Although there was no clear relationship between needle N concentrations and HWA densities within saplings, $A_{sat}$ varied with HWA density. In some studies, photosynthetic rates and herbivory are inversely related within plants, due to compensatory responses that reduce the effect of herbivory on carbohydrate production by reallocating resources, such as N, from affected to unaffected leaves (Pinkard et al. 2011). However, our needle N and protein concentration data were not consistent with a compensatory response to the HWA (Gómez et al. 2012), as there were no clear correlations between these variables and $A_{sat}$.

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Further, changes in $A_{sat}$ were not associated with changes in photosynthetic light response or respiration rates, suggesting that HWA effects on $A_{sat}$ did not result from direct effects on enzyme activity or light harvesting. Rather, our results suggest that HWA effects on $A_{sat}$ may have resulted from reductions in stomatal conductance, consistent with patterns observed in other studies (Domec et al. 2013, McDonald 2013, Gonda-King et al. 2014). $A_{sat}$ and $g_s$ varied in parallel with increasing HWA density, and there was a significant positive relationship between $A_{sat}$ and $g_s$, such that variation in $g_s$ accounted for 78% of the variation in $A_{sat}$. Because $g_s$ did not significantly vary with the needle-to-air vapor pressure deficit, changes in $g_s$ may have been driven by changes in plant–water relations, which we did not measure but have been observed to vary with HWA infestation (Gonda-King et al. 2012, Domec et al. 2013). Alternatively, changes in $g_s$ may have resulted from changes in $A_{sat}$, as $g_s$ may vary in response to changes in intercellular $CO_2$ concentrations associated with changes in $A_{sat}$ (Lewis et al. 2013). Regardless, our results suggest that the effect of the HWA on $A_{sat}$ was negative and independent of HWA effects on needle N concentrations.

In summary, HWA infestation was associated with changes in soil N cycling, and N allocation, needle biochemistry and physiology of eastern hemlock saplings, and the observed changes in individual variables generally were consistent with previous studies. However, our results suggest that the mechanisms regulating the relationships between HWA infestation and changes in ecosystem N cycling and the biochemistry and physiology of host trees may be scale dependent. Soil nitrification rates, $^{15}N$ uptake and needle N and protein concentrations were not correlated. Further, although needle N concentrations were greater in infested compared with uninfested saplings, HWA densities did not vary with needle N concentrations within saplings, suggesting that spatial variation in HWA densities within saplings was independent of variation in needle N concentration, and was the result of other factors, such as limitations on within-tree dispersal mechanisms. In turn, spatial variation in HWA densities within saplings may affect spatial patterns in $A_{sat}$ within infested saplings, and the potential negative effects of the HWA on $A_{sat}$ may be mediated through effects on $g_s$, as observed in other studies, rather than through effects on needle N concentration or other aspects of the photosynthetic machinery. The lack of clear linkages among soil N cycling, tree N dynamics and photosynthetic processes suggests that responses to the HWA at these scales are not tightly linked, possibly due to direct effects of the HWA on nitrogen uptake by host trees, as well as effects of other scale-dependent factors, such as changes in plant hydrology.

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## Conflict of interest

None declared.

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