ECOSYSTEMS

Impacts of Hemlock Loss on Nitrogen Retention Vary with Soil Nitrogen Availability in the Southern Appalachian Mountains

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ABSTRACT

The impacts of exotic insects and pathogens on forest ecosystems are increasingly recognized, yet the factors influencing the magnitude of effects remain poorly understood. Eastern hemlock (*Tsuga canadensis*) exerts strong control on nitrogen (N) dynamics, and its loss due to infestation by the hemlock woolly adelgid (*Adelges tsugae*) is expected to decrease N retention in impacted stands. We evaluated the potential for site variation in N availability to influence the magnitude of effects of hemlock decline on N dynamics in mixed hardwood stands. We measured N pools and fluxes at three elevations (low, mid, high) subjected to increasing atmospheric N deposition where hemlock was declining or absent (as reference), in western North Carolina. Nitrogen pools and fluxes varied substantially with elevation and increasing N availability. Total forest floor and mineral soil N increased ($P < 0.0001$, $P = 0.0017$, resp.) and forest floor and soil carbon (C) to N ratio decreased with elevation ($P < 0.0001$, $P = 0.0123$, resp.), suggesting that these high elevation pools are accumulating available N. Contrary to expectations, subsurface leaching of inorganic N was minimal overall (<1 kg ha^{-1} 9 months^{-1}), and was not higher in stands with hemlock mortality. Mean subsurface flux was 0.16 ± 0.04 (SE) (kg N ha^{-1} 100 days^{-1}) in reference and 0.17 ± 0.05 (kg N ha^{-1} 100 days^{-1}) in declining hemlock stands. Moreover, although subsurface N flux increased with N availability in reference stands, there was no relationship between N availability and flux in stands experiencing hemlock decline. Higher foliar N and observed increases in the growth of hardwood species in high elevation stands suggest that hemlock decline has stimulated N uptake and growth by healthy vegetation within this mixed forest, and may contribute to decoupling the relationship between N deposition and ecosystem N flux.

Key words: atmospheric nitrogen deposition; hemlock woolly adelgid; nitrogen flux; pest outbreaks; exotic species impacts; biotic sinks.

INTRODUCTION

The functional impacts of exotic insects and pathogens are increasingly recognized, with a growing number of studies documenting short- and long-term changes in forest structure and function as a result of pest- or pathogen-induced tree defoliation, loss of vigor, or death (Lovett and others

2006; Gandhi and Herms 2010). Short-term effects often include defoliation, changes in microclimate and/or light conditions, increases in coarse woody debris, and/or altered biogeochemical cycling and resource availability (Jenkins and others 1999; Kizlinski and others 2002; Ford and Vose 2007; Nuckolls and others 2009). Long-term consequences can include altered foliage and litter quality, changes in soil organic matter production and turnover, shifts in species composition, altered ecological interactions and ecosystem productivity (Stadler and others 2005a; Orwig and others 2008).

The factors influencing the magnitude of such effects have received much less attention. In their framework for understanding potential impacts of exotic pests and pathogens, Lovett and colleagues (2006) proposed organizing predictions around the features of the host species, namely its uniqueness in meaningful ecosystem processes such as seed production or nutrient cycling, its importance or dominance within a forest stand, and its phytosociology (that is, whether the host grows in pure or mixed stands, and its position in successional dynamics of the forest). Whereas the impacts of losing unique species have recently been considered (Ellison and others 2005), there has been little empirical work examining how impacts vary with species dominance or phytosociology. To date, most of the studies examining ecosystem consequences of exotic insect or pathogen outbreaks have focused on single species stands or mixed stands where the host species is dominant. However, many exotic invaders and their hosts have large ranges extending beyond a single region to forests that differ vastly in composition and phytosociology.

One such example is the Eastern hemlock (*Tsuga canadensis*), which has a distribution that extends from the southern Appalachians to southern Canada and west to the central Lake states, and is currently declining throughout much of its eastern range due to infestation by an aphid-like insect native to Asia (Ellison and others 2005). Hemlock occurs as a dominant component in stands of the northeast and a co-dominant or minor constituent in mixed hardwood forests elsewhere. It is considered a foundation species because it exerts strong control on community dynamics and ecosystem function by regulating food webs, productivity, and the flux of water, energy and nutrients (Ellison and others 2005). Previous studies conducted in monotypic hemlock stands show that loss of these influential trees can decrease nitrogen retention and potentially redefine ecosystem structure and function for decades or longer (Jenkins and others 1999; Kizlinski and others 2002; Yorks and others

2003; Stadler and others 2005b; Orwig and others 2008). However, in the mixed hardwood forests of the southeastern US, loss of hemlock may not have the large impacts observed in monotypic stands of the Northeast. Knoepp and others (2011) found no differences in N mineralization rates or soil solution N concentrations between plots without hemlock and plots infested with hemlock woolly adelgid (HWA) and concluded that, as hemlock is replaced with hardwoods, nutrient cycling rates and processes in areas with hemlock will become similar to that in hardwood-only areas.

In addition to the factors proposed by Lovett and others (2006), there can exist substantial differences in abiotic conditions that may influence ecosystem response to pest- or pathogen-induced species decline. Anthropogenic increases in biologically reactive N in the biosphere, for example, have resulted in measurable changes in soil and forest floor N pools and C: N ratios (Aber and others 2003). Within regions differences in these pools can be pronounced because N deposition varies with elevation and topography, as well as proximity to industrial areas (Weathers and others 2000, 2006). In a study examining the impacts of eastern hemlock mortality in sites with differing rates of N deposition, Templer and McCann (2010) found that biota take up and transform much of the N found in soil solution when external N inputs are high but not when N inputs are low. These findings suggest that both biotic and abiotic factors have a role in mitigating the impacts of tree mortality resulting from exotic invasion; however, the interactive effects of such factors remain largely uncharacterized. The loss of a species that strongly regulates nutrient retention may have a greater impact on nutrient dynamics in stands with high initial N availability versus more N-limited stands. In contrast, biotic demand for nutrients may reduce the impact of species loss on nutrient retention regardless of exogenous inputs. As exotic introductions accelerate (Levine and D'Antonio 2003), there is a critical need to understand controls on invasive species impacts to anticipate how forest ecosystems will change in the future.

The purpose of this study was to investigate potential abiotic and biotic controls on the biogeochemical impacts of an exotic insect. We evaluated N pools and fluxes in southern Appalachian forest stands subjected to increasing rates of N deposition where hemlock was absent (reference) or declining due to HWA to test the following hypotheses: (1) hemlock loss will decrease ecosystem N retention, with effects being less severe in the southern Appalachians compared to those reported for more

northern forests because hemlock is sub-dominant and co-occurs with hardwoods not susceptible to attack by HWA, and (2) greater N availability will lower relative biotic demand for N resulting in larger subsurface N fluxes in stands experiencing hemlock decline.

METHODS

Site Description and Selection

This study was conducted at Coweeta Hydrologic Laboratory, a USDA Forest Service experimental forest in the southern Appalachian mountains of western North Carolina, USA. Annual precipitation varies with elevation, with stands receiving 1800 mm at low elevations and 2400 mm at high elevations. Rainfall is evenly distributed throughout the year with greater than 100 mm occurring in most months. The growing season extends from early May to early October. Mean monthly temperatures are highest in June through August ($\sim$20°C) and lowest in December through January ($\sim$5°C) (Knoepp and others 2008). Soils at Coweeta are mostly Inceptisols or Ultisols, classified as Mesic or Humic Hapludults, or Typic Humudepts. Parent material consists of high-grade metamorphic rocks (that is, mica gneiss, mica schist) and metasedimentary rocks (that is, metasandstone, phyllite, shale).

Prior to its acquisition by the Forest Service in 1934, areas within the Coweeta basin were burned semiannually by the Cherokee Indians, used for grazing or agriculture, and logged for *Castanea dentata* (Elliott and Vose 2011). Today, the principal overstory taxa include *Quercus*, *Carya*, and *Liriodendron* with scattered groups of *P. rigida* and *T. canadensis* (Elliott and Vose 2011). Evergreen shrubs (*Rhododendron* and *Kalmia*), along with midcanopy species *Cornus*, *Robinia*, *Acer*, and *Betula* form a dense understory cover (Day and others 1988; Elliott and Vose 2011). HWA was first documented in the Coweeta basin in 2003, with extensive infestation by 2005 (Nuckolls and others 2009). Tree mortality can occur in as little as 4 years (McClure 1991), and nearly 50% mortality of eastern hemlocks found within Coweeta Hydrologic Laboratory was observed in 2010 (Ford and others 2011). All eastern hemlocks measured in this study were still standing, but visually estimated to have lost 95–100% of their canopy.

We established six 20 m $\times$ 20 m (0.04 ha) study plots at each of three elevations, low (698–716 m), mid (917–943 m), and high (1,402–1,433 m), experiencing increasing rates of atmospheric N deposition ($n = 18$ plots total) (Table 1). Plots at each elevation had a similar composition of mixed hardwoods, but half of them contained several ($n = 5$–31) dead or dying eastern hemlock trees (declining hemlock; DH) and the other half contained no hemlock trees and were used as reference (REF) (Table 1). Plots across the elevation gradient with hemlock unaffected by HWA would have been an ideal reference, but there were no stands containing hemlock unaffected by HWA. Only stands lacking or with very small amounts of *Rhododendron maxima* were selected to avoid biasing nutrient cycling measurements (Boettcher and Kalisz 1990).

Vegetation Measurements

To characterize the vegetation in each plot, we measured the diameter of all trees at least 2.5 cm at DBH (1.37 m above the forest floor) and identified them to species with the exception of *Carya* spp., which were identified to genus. We also counted all seedlings (<0.5 m) and saplings (>0.5 m) within a 1 m $\times$ 20 m section on the west side of each plot.

We collected foliage samples from the mid-crown of the three dominant hardwood species in each plot (determined by total basal area) using a slingshot and/or pole pruner between 21 July 2010 and 4 August 2010 (Appendix 1 in Supplementary Material). Within each plot, five individuals per species were sampled; if fewer individuals of a species existed in the plot, then we sampled the individuals present. Samples were composited by species, air-dried, and ground to less than 1 mm using a Wiley mill, then a sub-sample was taken for chemical analysis. Total C and N were determined by combustion on an Elementar Flash EA 1112 CN analyzer (Thermo Scientific, Waltham, MA). Following the approach of Ollinger and others (2002), we scaled foliar N concentration to the plot-level by calculating the sum of foliar N concentrations among sampled species, weighted by the relative foliage mass of each species within a plot. This weighting technique allowed for comparison of concentration patterns with respect to elevation and vegetation type despite variation in species composition across plots.

In October 2010 we collected increment cores from the same individuals from which we collected foliage samples to calculate basal area increment (BAI) (Appendix 1 in Supplementary Material). Annual ring widths were measured to the nearest 0.001 mm using a Velmex Unislide (Velmex Inc., Bloomfield, NY) and an Olympus SZ40 Stereozoom microscope (Olympus America Inc., Center Valley,

Table 1. Site Characteristics for the Declining Hemlock (DH) and Reference (REF) Plots at Low (L), Mid (M), and High (H) Elevations, Including Dominant Vegetation, Elevation (m), Mean ($\pm$ SE) Atmospheric Wet Inorganic Nitrogen Deposition (NH_4-N + NO_3-N) for April 2010–December 2010, Hemlock (*Tsuga canadensis*) % Basal Area per Plot, and Mean ($\pm$ SE) Seedling and Sapling Densities

Site	Dominant vegetation	Elevation (m)	Hemlock % BA within plot	N dep. (kg ha^{-1} 9 months^{-1})	Vegetation density (seedlings m^{-2})	(saplings m^{-2})
DH-L	*Acer rubrum, Betula lenta, Ilex opaca, Liriodendron tulipifera, Nyssa sylvatica, Oxydendrum arboretum, Pinus rigida, Quercus alba*	710–716	23–56	3.45 $\pm$ 0.06	12.68 $\pm$ 3.42	0.50 $\pm$ 0.22
REF-L	*A. rubrum, B. lenta, Carpinus caroliniana, Fagus grandifolia, L. tulipifera, Q. alba, Quercus velutina*	698–715	0	3.40 $\pm$ 0.06	6.10 $\pm$ 3.02	1.13 $\pm$ 0.58
DH-M	*A. rubrum, B. lenta, Carya* spp., *L. tulipifera, N. sylvatica, O. arboretum*	917–934	13–52	3.96 $\pm$ 0.20	8.07 $\pm$ 1.74	0.72 $\pm$ 0.25
REF-M	*A. rubrum, Carya* spp., *L. tulipifera, O. arboretum, Quercus coccinea, Q. velutina Robinia pseudoacacia*	924–943	0–4	4.12 $\pm$ 0.22	7.50 $\pm$ 1.83	2.72 $\pm$ 0.41
DH-H	*Acer pensylvanicum, A. rubrum, Betula alleghaniensis, Fagus grandifolia Quercus rubrum*	1402–1433	5–8	4.44[1]	6.83 $\pm$ 2.80	0.37 $\pm$ 0.19
REF-H	*A. pensylvanicum, A. rubru, Acer saccharum, B. alleghaniensis, Ostrya virginiana, Prunus serotina*	1411–1423	0	4.44[1]	4.80 $\pm$ 1.21	0.22 $\pm$ 0.04

[1]*A standard error could not be calculated for these sites because only one rain gauge was available. Basal area and foliar nitrogen concentrations of dominant hardwood species are given in Appendix 1 in Supplementary Material.*

PA) interfaced with Measure J2X software. We determined annual BAI from the difference of successive annual growth rings dating back to 1991 (14 years pre-infestation). BAI of all trees was expected to be highly dynamic among years, and inter-annual variability in tree growth is highly reflective of climatic conditions (Fritts 1972; Ford and others 2011). To isolate differences due to N availability and minimize those of climate, we followed the approach of Nuckolls and others (2009) and Ford and others (2011) and calculated the ratio of mean BAI in DH versus REF plots (mean BAI in DH [cm^2]/mean BAI in REF [cm^2]) for each species at each elevation.

Forest floor (O horizons + decaying wood) was sampled on all plots in March 2010 for determination of total mass and pools of C and N. We collected five samples per plot with a 0.09 m^2 quadrat. Samples were oven-dried, weighed, and processed prior to analysis for total C and N by combustion as described above.

Soil and Environmental Measurements

We collected composite soil samples from each plot in June 2010. Samples were collected at depths of 0–10 and 10–30 cm using a 2.0-cm diameter soil probe, and were made up of 20–25 individual samples per plot. Samples were stored in plastic bags and placed in coolers on ice during transport to the laboratory. Soils were air-dried, mixed thoroughly, and sieved (<2 mm) prior to subsampling for chemical analysis. Bulk density, both total and smaller-than-2 mm fractions, was determined for soils on each plot by collecting five 4.3-cm diameter PVC cores per plot at each of two depths, 0–10 and 10–30 cm. These data were used to calculate soil nutrient pools in kg ha^{-1}. Soil

chemical analyses included total C and N by combustion (as above) and 0.01 M CaCl$_2$ soil pH.

Ion-exchange resin lysimeters (IERs) (Susfalk and Johnson 2002) were installed to collect inorganic N from forest floor and subsoil leachate. The IERs were constructed by placing 25 g of mixed-bed resin (Rexyn #R208-500) into a 5.08-cm PVC coupling between two Nitex® nylon sheets, which were then sandwiched between two layers of nutrient-free silica play sand. Eight IERs were randomly located throughout each plot: four below the forest floor (''forest floor lysimeters'') and four below the major rooting zone (''mineral soil lysimeters''). To place the four mineral soil lysimeters, we dug two 50-cm deep pits each with two channels extending perpendicularly from the bottom. One IER was placed at the end of each channel. Channels were long enough to ensure that the soil column above the lysimeters remained undisturbed. Fresh IERs were deployed in the field during 2010 for three consecutive 100-day periods beginning April 10, July 23, and October 27, corresponding with the early growing season, late growing season, and fall/dormant season in the southern Appalachians. At the time of collection, IERs were stored in individual plastic bags, and kept cool during transport to the laboratory. Resin was extracted using 2 M KCl and the extract was filtered through 0.7 μm Whatman filter paper and frozen until analysis (Langlois and others 2003). Extracts were analyzed for NH$_4$-N using the phenolate method and NO$_3$-N using a cadmium column reduction on a Lachat QuikChem 8000 (Hach Company, Loveland, CO). Following correction for lab and field blanks, we determined the mass of N in the resin by multiplying extract concentration by the volume of KCl used for extraction. Flux was calculated on a per area basis by dividing N mass in leachate by lysimeter collection area (20.4 cm^2).

Total wet inorganic N deposition (NH$_4$-N + NO$_3$-N) was calculated using precipitation amount (cm) and chemistry data from Coweeta rain gauges SRG19 and 20 for low elevation sites, SRG2, 40, and 45 for mid-elevation sites, and SRG 31 for high elevation sites (Knoepp and others 2008) (Table 1). Dry deposition and cloud water deposition were not estimated for these sites, but are likely to increase substantially with elevation in mountainous terrain (Lovett and others 1997; Weathers and others 2006; Knoepp and others 2008). We determined percentage N retention by subtracting inorganic N outputs based on the amount of N leached from the soil at 50 cm and captured with mineral soil lysimeters from inorganic N inputs based on precipitation chemistry and dividing by inorganic N

inputs. We also measured mineral soil moisture and temperature 5 cm below the soil surface using an ECH$_2$O Water & Temp Sensor (Decagon Devices, Pullman, WA) at each plot during quarterly sampling (2009: October; 2010: January, April, July, October; 2011: February) except at high elevations during the winter when the top 5 cm was frozen and the probe could not be inserted.

Statistical Analyses

Samples with a single collection date (foliage, forest floor, composite soil samples) were analyzed with ANOVA using elevation (low, mid, high) and vegetation type (DH, REF) as main and interactive (elevation × vegetation type) effects. For resin-based data with repeated collection dates, we used plot means for each temporal period and determined changes over time with a repeated measures statement using an unstructured variance–covariance matrix in a linear mixed model with plot (elevation × disturbance) as the subject. For soil moisture and temperature, we used an auto-regressive and heterogeneous auto-regressive (respectively) covariance structure to account for temporal autocorrelation. For all models, we used the Slice statement to evaluate simple main effects of elevation and vegetation type in the elevation × vegetation type interaction. Simple main effects test the null hypothesis of no effect of factor A at each level of factor B separately and vice versa (Quinn and Keough 2002). Thus, we examined the effect of hemlock decline on N pools and fluxes at each elevation separately, as well as differences in N pools and fluxes along the elevation gradient for each vegetation type separately. For BAI ratios, we calculated the slope of pre-infestation (1991–2004) and post-infestation (2005–2010) growth using linear regression, and tested these slopes for differences between time periods and among elevation classes using linear contrasts in a generalized linear model. We used the same approach to evaluate whether slopes differed with time period (pre and post) at each elevation separately. Data were logarithmically transformed as necessary to meet assumptions of normality and equal variance. All analyses were conducted in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Wet inorganic N (NO$_3$-N + NH$_4$-N) deposition increased with increasing elevation ($F_{2,18} = 34.4$, $P < 0.001$) from April 2010 to December 2011 (Figure 1). Total wet inorganic N deposition ranged
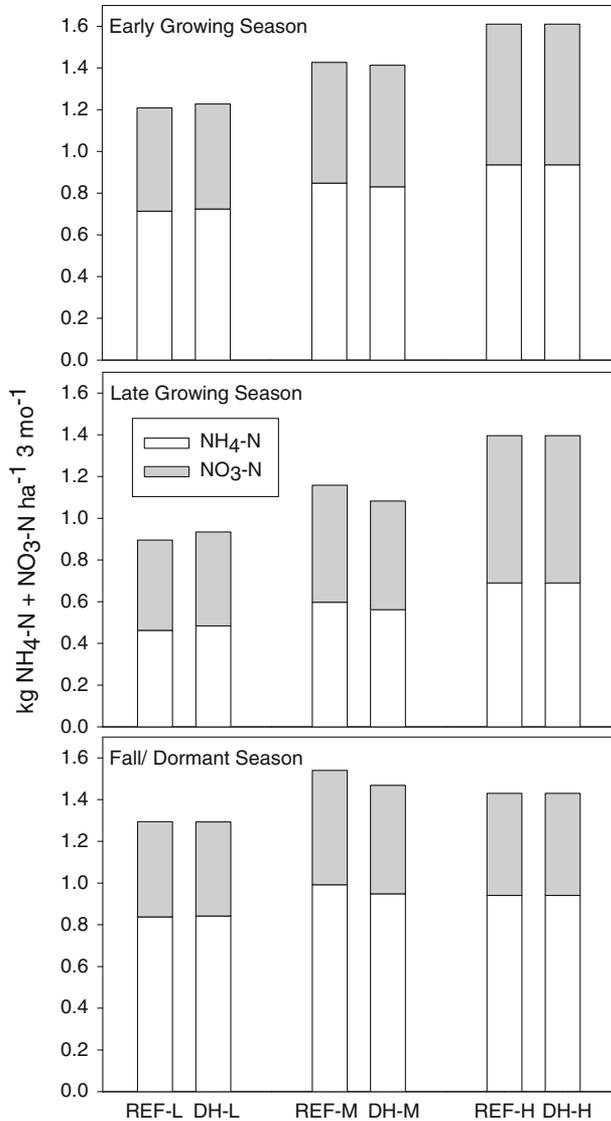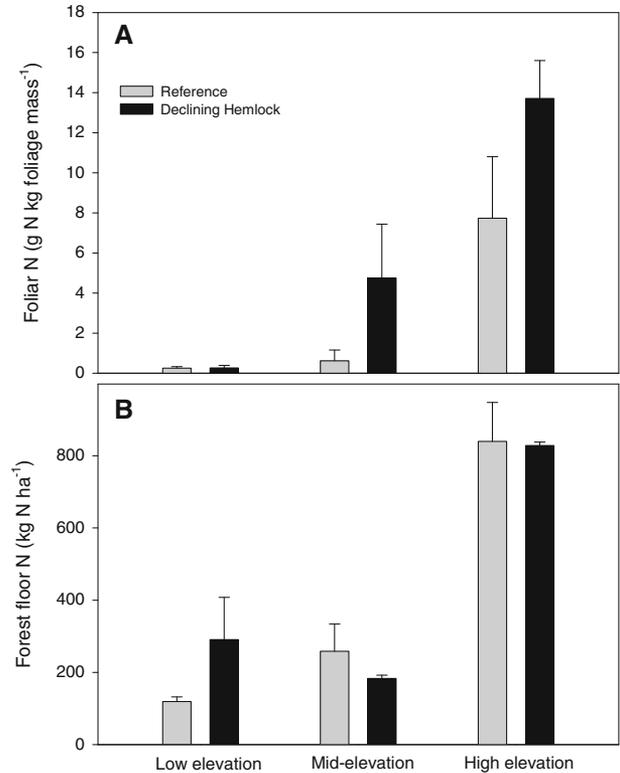
Figure 1. Wet inorganic nitrogen deposition for 3 month periods starting April (early growing season), August (late growing season), and November (fall/dormant) 2010, corresponding to in situ sampling. Refer to Table 1 for site code explanations.

Figure 2. Plot-scale foliar nitrogen concentration of overstory hardwood trees weighted by relative foliage mass (**A**) and total forest floor (Oi + Oe + Oa + wood) nitrogen content (**B**). The species sampled for foliar chemistry are listed in Appendix 1 in Supplementary Material. Values are means across plots ± 1 SE.

from 3.40 ± 0.06 (mean $\pm$ SE) kg ha^{-1} in low elevation plots to 4.44 kg ha^{-1} (based on one rain gauge) at high elevation plots (Table 1). Soil moisture did not differ by elevation or between DH and REF plots over the sampling period. Soil temperature, however, declined with increasing elevation ($F_{2,54} = 15.6$, $P < 0.001$) and was lower in DH than REF plots ($F_{1,53} = 12.8$, $P < 0.01$).

Vegetation Measurements

Seedling density generally declined with increasing elevation (Table 1), averaging 9.4 ± 2.5 ($\pm$ SE) in low elevation plots, 7.8 ± 1.4 in mid, and 5.8 ± 1.4 seedlings m^{-2} in high elevation plots. However, these differences were not significant, nor was there an effect of vegetation type or an elevation × vegetation type interaction. Mid-elevation plots exhibited a higher sapling density (0.72 ± 0.25 saplings m^{-2}) than low (0.50 ± 0.22) or high (0.37 ± 0.19) elevation plots ($F_{2,18} = 5.3$, $P = 0.02$), but there was no difference between DH and REF plots, and no interaction.

The mass-based foliar N concentration of hardwood species increased with elevation ($F_{2,18} = 17.5$, $P < 0.001$) and was higher in DH plots across all elevations ($F_{1,18} = 5.0$, $P = 0.04$) (Figure 2A). Although the interaction between elevation and vegetation type was not significant, analysis of the simple main effect of vegetation type at each elevation indicated that the effect of hemlock decline on foliar N was not equivalent along the gradient. At high elevations, foliar N concentration was significantly greater in DH than REF plots ($F_{1,6} = 5.2$, $P = 0.04$), whereas at low- and mid-elevations there was no difference. Testing for differences in

foliar N with elevation at each level of vegetation type, we likewise found a more appreciable change in DH than REF plots. That is, there was a larger increase in foliar N concentration with elevation among DH plots ($F_{2,9}$ = 13.7, P < 0.001) than among REF plots ($F_{2,9}$ = 5.19, P = 0.02) (Figure 2A).

The mass of total N in the forest floor (Oi + Oe + Oa + wood) also increased with elevation ($F_{2,18}$ = 49.4, P < 0.001); although there was no effect of elevation on individual forest floor horizons. No differences were observed between DH and REF plots, and the interaction of elevation × vegetation type was not significant. Mean forest floor N was 834 ± 48.6 kg ha^{-1} (± SE) in high elevation, 221 ± 37.4 kg ha^{-1} in mid-elevation, and 205 ± 65.0 kg ha^{-1} in low elevation plots (Figure 2B). Mean forest floor C pools were 19.7 ± 1.29 Mg ha^{-1} in high elevation, 8.52 ± 1.05 Mg ha^{-1} in mid-elevation, and 10.3 ± 3.44 Mg ha^{-1} in low elevation plots (data not shown). This corresponded with a decrease in the total forest floor C:N molar ratio ($F_{2,18}$ = 52.5, P < 0.001), which averaged 28 in high elevation, 46 in mid-elevation, and 58 in low elevation plots (data not shown). However, there were no differences in forest floor C:N between vegetation types, and the elevation × vegetation type interaction was not significant.

The relationship between the BAI ratios of hardwood trees and time (years) varied among species and elevations, and differed between the pre- and post-infestation periods (Figure 3). Prior to HWA infestation, there was a positive significant relationship between BAI ratio and time for *Oxydendrum arboreum* in mid-elevation plots (B_1 = 0.030, $t_{1,14}$ = 5.05, P < 0.001) and *Acer rubrum* in high elevation plots (B_1 = 0.093, $t_{1,14}$ = 2.88, P = 0.01); whereas significant negative relationship were observed for *Betula lenta* (B_1 = −0.058, $t_{1,14}$ = −4.19, P = 0.01) and *Liriodendron tulipifera* (B_1 = −0.065, $t_{1,14}$ = −6.43, P < 0.001) in low elevation plots. There was no relationship between BAI ratio and time during the pre-infestation period for the other species sampled. Post-infestation, we detected strong positive trends between BAI ratio and time for all of the species sampled in high elevation plots (*Acer pensylvanicum*: B_1 = 0.050, $t_{1,14}$ = 3.66, P = 0.02; *Acer rubrum*: B_1 = 0.164, $t_{1,14}$ = 2.03, P = 0.11; *Betula alleghaniensis*: B_1 = 0.119, $t_{1,14}$ = 2.38, P = 0.07). There were negative trends between BAI ratio and time for *Betula lenta* in low elevation plots (B_1 = −0.045, $t_{1,14}$ = −2.11, P = 0.10) and *Oxydendrum arboreum* in mid-elevation plots (B_1 = −0.040, $t_{1,14}$ = −2.44, P = 0.07).
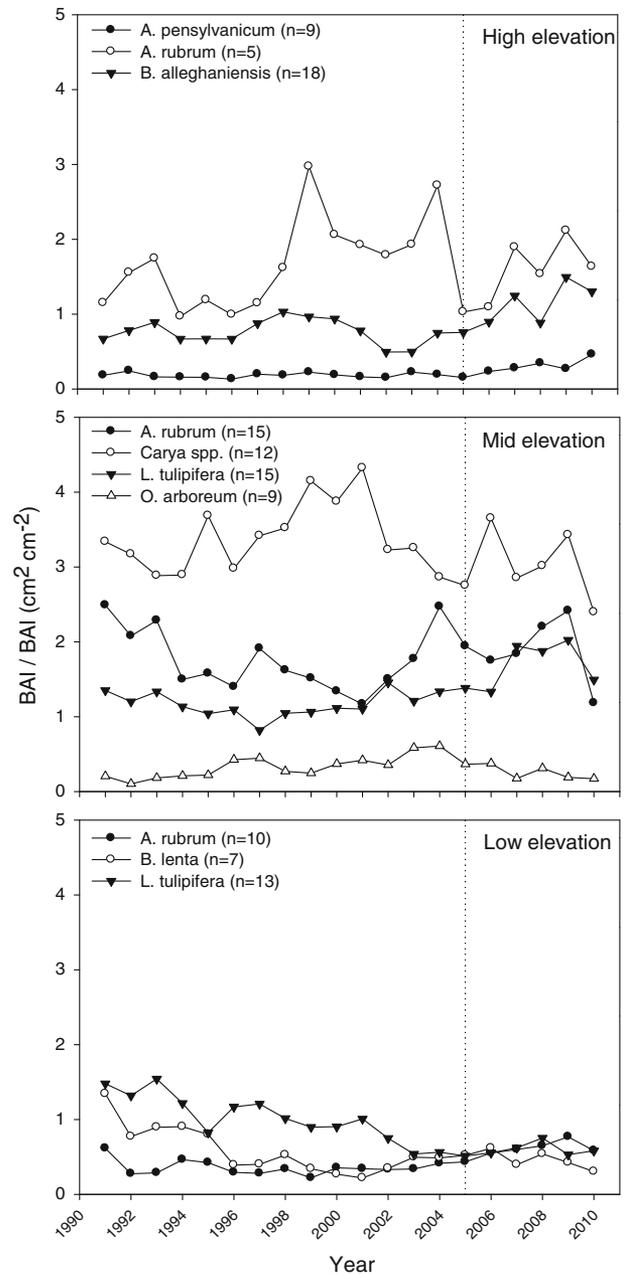

Figure 3. Mean ratio of annual basal area increment (BAI) of hardwood species in declining hemlock stands relative to the BAI of hardwood trees in nearby reference stands. The species and sample size per elevation class are given in the legend. *Dashed lines* mark the year when hemlock stands were infested by hemlock woolly adelgid.

For the other species sampled, there was no relationship between BAI ratio and time during the post-infestation period.

Comparison of slopes calculated from BAI ratios showed that, prior to infestation, tree growth was similar across DH plots at different elevations (Figure 3). Post-infestation, however, growth

diverged among elevation classes ($F_{2,20} = 6.65$, $P < 0.01$). Slopes were strongly positive in high elevation stands but close to zero or negative in mid- and low-elevation plots. Comparisons of slopes pre- versus post-infestation at each elevation indicated that tree growth increased at high elevations ($F_{1,18} = 4.27$, $P = 0.05$), but remained similar at mid- and-low elevations following HWA infestation.

Soil and Environmental Measurements

Similar to forest floor N, total soil N increased with elevation in both the 0–10 cm ($F_{2,18} = 11.34$, $P < 0.01$) and 10–30 cm ($F_{2,18} = 23.0$, $P < 0.001$) depths, but did not differ between DH and REF plots at either depth. The top 10 cm of soil contained $1,536 \pm 106.1$ (mean $\pm$ SE) kg N ha^{-1} and $25,493 \pm 1,264$ kg C ha^{-1} in low elevation; $1,429 \pm 173.7$ kg N ha^{-1} and $27,262 \pm 2,929$ kg C ha^{-1} in mid-elevation; and $2,712 \pm 213.4$ kg N ha^{-1} and 43,319 kg C ha^{-1} in high elevation plots (data not shown). The average soil C:N

(molar) ratio in the 0–10 cm depth was 19, 23, 20 in low, mid, and high elevation plots, respectively ($F_{2,18} = 6.48$, $P = 0.01$; data not shown). The interaction term was not significant.

Solution entering the soil profile from just below the forest floor was dominated by nitrate-N (NO_3-N) during the late summer and fall/dormant periods but there were equal amounts of ammonium-N (NH_4-N) and NO_3-N during the early growing season (Figure 4). Elevation and season were important factors governing forest floor NO_3-N flux, which increased with elevation ($F_{2,54} = 9.79$, $P < 0.01$) and was significantly greater late in the growing season (temporal period: $F_{2,54} = 49.0$, $P < 0.001$; Tukey HSD: $P < 0.001$). NO_3-N flux from the forest floor did not differ with vegetation type over the sampling period, and there was no interaction between vegetation type and elevation.

In contrast, total inorganic N flux (NO_3-N + NH_4-N) from the forest floor depended on elevation, time, and vegetation type (three-way interaction: $F_{4,54} = 2.99$, $P = 0.06$; Figure 4). In DH plots, forest floor inorganic N flux increased with elevation
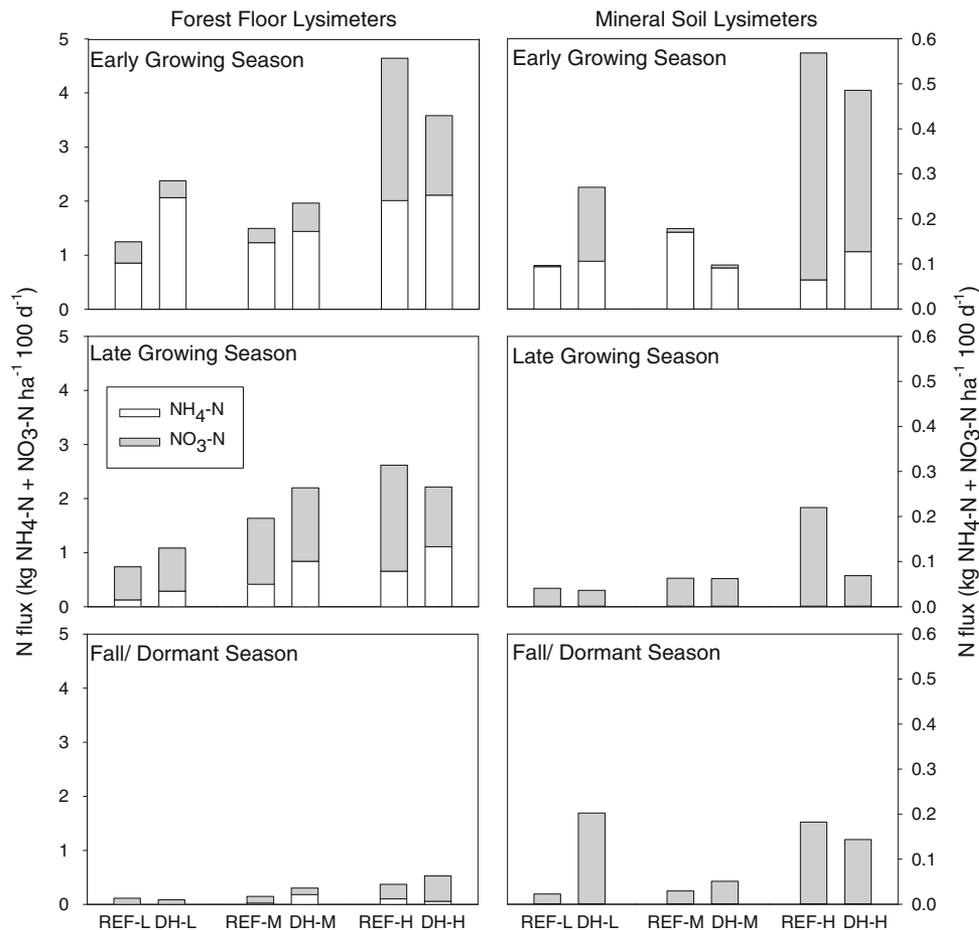
Figure 4. Fluxes of nitrate-N and ammonium-N from the forest floor (*left panel*) and mineral soil (*right panel*) in reference (REF) and declining hemlock (DH) plots at three elevations (L = low, M = mid, H = high) for the 9-month sampling period, April 2010–February 2011. Lysimeters were in situ three consecutive 100-day periods, beginning April 10, July 23, and October 27, corresponding with the early growing season, late growing season, and fall/dormant season in western NC. Values shown are means across plots (N = 3 plots per elevation × vegetation combination).

during the fall/dormant period ($F_{2,27} = 8.17$, $P = 0.006$), but was similar across elevations during other periods ($P > 0.10$). In REF stands, however, inorganic N flux increased with elevation during the early growing season ($F_{2,27} = 11.5$, $P = 0.002$), but not during other periods. Across all temporal periods, vegetation type modulated the effect of elevation on inorganic N flux (elevation × vegetation type: $F_{2,54} = 3.29$, $P = 0.07$), such that the simple main effect of elevation on total inorganic N flux was appreciably stronger in REF plots ($F_{2,27} = 24.1$, $P < 0.001$) than in DH plots ($F_{2,27} = 5.65$, $P = 0.02$).

Most N leached from the mineral soil was in the form of NO_3-N, but this amounted to less than 0.9 kg ha^{-1} at any one elevation over the entire 300-day sampling period. Although NO_3-N flux varied with elevation ($F_{2,54} = 2.96$, $P = 0.09$) and temporal period ($F_{2,54} = 3.41$, $P = 0.07$), trends were inconsistent and differed for each vegetation type (Figure 4). In REF plots, NO_3-N flux increased significantly with elevation during the early and late growing seasons ($F_{2,27} = 4.57$, $P = 0.03$; $F_{2,27} = 5.44$, $P = 0.02$, resp.) but not during the fall/dormant period. Conversely, NO_3-N flux did not vary significantly with elevation in DH plots during any temporal period ($P \geq 0.20$ for all combinations).

Total inorganic N fluxes were also relatively small (<1 kg ha^{-1}) at any given elevation during the sampling period. Although trends differed with temporal period ($F_{2,54} = 53.6$, $P < 0.001$), similar to NO_3-N, the relationship between inorganic N flux, elevation, and time varied with vegetation type (Figure 4). Inorganic N flux generally increased with elevation in REF plots throughout the sampling period ($P < 0.17$ for all combinations), although trends were only significant during the late growing season ($F_{2,54} = 5.53$, $P = 0.02$). In DH plots, however, there was no apparent effect of elevation during any temporal period ($P \geq 0.37$ for all combinations) (Figure 5).

The percentage inorganic N retained in REF and DH plots ranged from 62 to 98% and was similar when averaged over elevation classes (REF: $89\% \pm 0.03$ SE; DH: $87\% \pm 0.05$). However, there was a trend for lower inorganic N retention in REF plots than DH plots at high elevations, and higher inorganic N retention in REF plots than DH plots at low elevations, mainly attributable to differences in the retention of NO_3 among these plots (Figure 6). Analysis of simple main effects confirmed that NO_3 retention was more strongly associated with elevation in REF plots ($F_{2,18} = 2.94$, $P = 0.09$) than in DH plots ($F_{2,18} = 0.38$, $P = 0.69$).
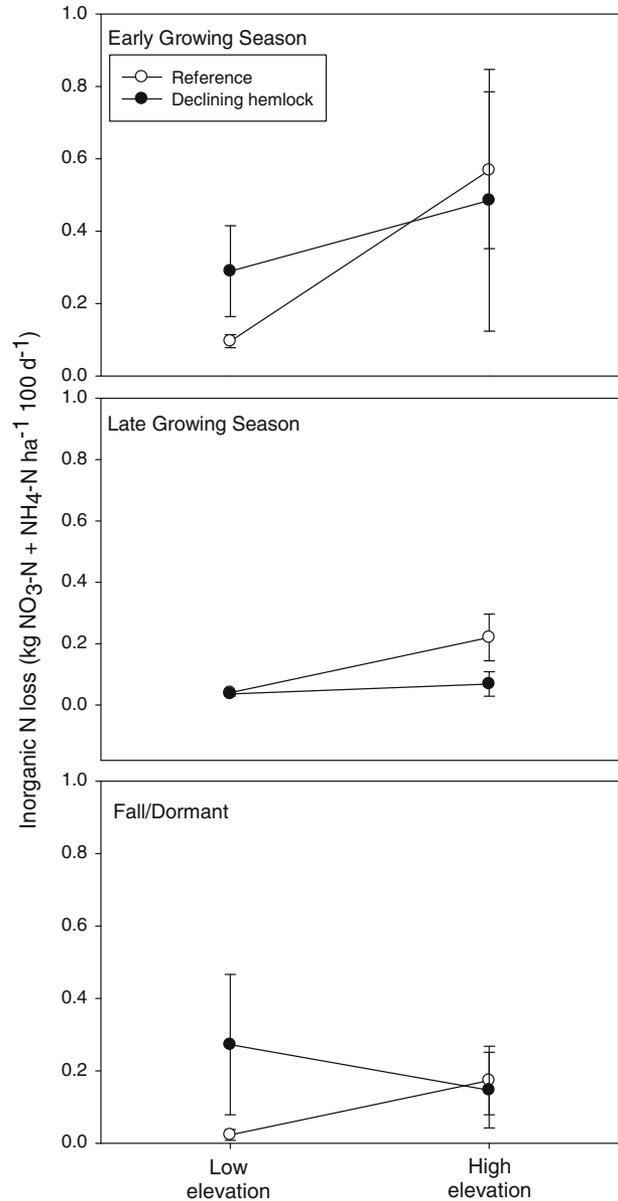

Figure 5. Interaction plots of subsurface inorganic N flux from low and high elevation stands during each sampling period.

DISCUSSION

We organize our discussion relative to the two hypotheses tested in this study.

Hypothesis 1 Hemlock loss will decrease ecosystem N retention; effects of hemlock loss will be less severe in the southern Appalachians compared to those reported for more northern forests.

To date, most studies evaluating the impacts of hemlock decline on N cycling have been conducted in New England, where hemlock occurs in
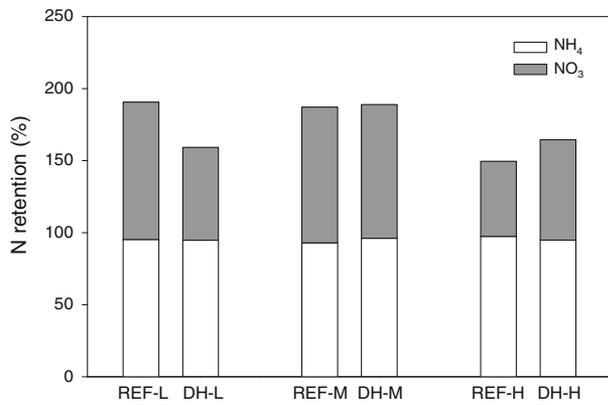
Figure 6. Nitrogen retention by elevation and vegetation type calculated as (N input–N output)/N input, where N input is based on wet inorganic atmospheric N deposition and N output is based on observed inorganic N flux determined with resin-based lysimeters installed in the mineral soil at 50-cm depth. Refer to Table 1 for site code explanations.

monotypic stands or as a dominant species in mixed stands. These studies demonstrate that nitrification rates increase in stands infested with HWA regardless of hemlock percent mortality and within 2–3 years of infestation (Jenkins and others 1999; Kizlinski and others 2002; Orwig and others 2008). Nitrogen leaching is thus expected to increase although ecosystem N retention is expected to decrease following hemlock decline (Jenkins and others 1999). Indeed, Yorks and others (2003) observed elevated nitrate concentrations in soil solutions within 2–3 months of girdling hemlocks to simulate HWA-induced mortality; concentrations remained high relative to control stands for the duration of the study. In our study, the percentage of plot basal area accounted for by hemlock ranged from 5 to 56% across our study area (Table 1), and overall impacts on inorganic N retention were small. This result is consistent with that of Knoepp and others (2011a), who found minor effects of HWA infestation on N mineralization rates and soil solution inorganic N concentrations in southern Appalachian forests where hemlock represented less than 50% of plot basal area. Thus, the greater the relative importance of hemlock in New England versus southern Appalachian forests likely explains why the reported impacts of hemlock loss on N retention are more severe in the Northeast. This interpretation is also consistent with the hypothesis that the impacts of the loss of a given species on ecosystem processes will depend on its relative importance within a forest (Lovett and others 2006).

A related question that remains difficult to address is how impacts scale with species importance.

Although hemlock plays an important ecological role in forests, particularly with respect to N cycling (Lovett and others 2004; Templer and others 2005), we found no evidence for a linear relationship between declining hemlock basal area and ecosystem N retention (data not shown). Given the contrasting impacts of hemlock loss in New England versus the southern Appalachians, we might expect a non-linear relationship, with a threshold value of hemlock relative importance at which changes in ecosystem N retention become profound. More research will be needed to understand how the biogeochemical impacts of tree species loss vary with species abundance and structural and functional roles.

Hypothesis 2 Greater N availability will lower relative biotic demand for N, resulting in larger subsurface fluxes of N in stands experiencing hemlock decline.

The coincident increases in wet atmospheric N deposition and the sizes of soil and vegetation N pools with elevation suggest that southern Appalachian forests are assimilating more N in response to enhanced N availability. We expected that increasing N availability would increase subsurface N fluxes as soil and biotic sinks became N saturated, yet the observed pattern of low mineral soil N flux at all sites suggests that these sinks have not yet reached capacity. More surprisingly, we also found no evidence that hemlock decline enhanced subsurface N fluxes, even in the high N sites. Although there was a trend for higher flux rates in DH plots than REF plots at low elevations, high elevation DH plots retained more N than REF plots at similar elevations (Figure 5). Moreover, DH plots at high elevations where N inputs were highest had nearly two times greater foliar N concentrations on a per foliage mass basis than reference stands at the same elevation, and hardwood trees increased their rate of growth following HWA infestation (Figure 3). Collectively, these results suggest that the strength of the biotic N sink has increased in high elevation stands with declining hemlock to the extent that available N is being taken up and assimilated into biomass rather than leached from the system.

We did not measure dissolved organic nitrogen (DON) flux, which could partly explain why we did not detect as much N leaching from soil solution as hypothesized. Qualls and others (2000) found that the flux of DON was 33 mg m^{-2} y^{-1} at 30–50 cm soil depth in similar southern Appalachian forests. However, previous work suggests that hemlock decline may lead to a decrease in DON flux from the soil solution. Yorks and others (2003) found

that mineral soil DON flux was 73–88% lower than nitrate flux in girdled hemlock stands, whereas DON and nitrate fluxes were similar in control stands with approximately 45% hemlock by basal area. These findings suggest that we may have underestimated N flux in our reference stands, but not necessarily our declining hemlock stands. Future studies should examine fluxes of DON and inorganic N following hemlock loss to better characterize ecosystem response.

Ecosystems with greater N inputs typically have greater N flux rates (van Breemen and others 2002). The interaction plots (Figure 5) demonstrate that N leaching is two to four times higher in high elevation reference plots than low elevation reference stands across all temporal periods. In contrast, the change in N leaching between low and high elevation DH plots is comparatively smaller and the direction of the change varies with temporal period: positive during early growing season, neutral during late growing season, and negative during the fall/dormant period. Nitrogen leaching in reference plots strongly mirrors the precipitation-driven N deposition pattern, in which N inputs are highest during early summer when precipitation is greatest and lowest during fall and winter when there is less precipitation. In high elevation DH plots, however, there is a decoupling of the relationship between N deposition and subsurface N flux. The observed trends are broadly consistent with the phenology of photosynthetic activity in this system. For example, during the April–July period, similarly high rates of subsurface N flux in high elevation declining and reference plots may be attributable to the late onset of photosynthetic activity combined with high N deposition rates. During the July–October period, photosynthetic activity in high elevation stands should promote N uptake, resulting in relatively lower subsurface N flux in declining than reference stands. Likewise, low levels of leaching in high elevation reference and declining stands during the October–February period may be due to low deposition rates and low biotic demand of surrounding vegetation, whereas the higher-than-expected fluxes at low elevations during this period may be due to the large canopy gaps left by declining hemlock, which may allow sunlight to reach the forest floor, thus encouraging faster snowmelt and greater N flushing (Jenkins and others 1999). Experiments will be needed to test the generality of these conclusions and reveal the mechanisms underlying these patterns. Yet, our results are comparable to those of others. In a study examining impacts of eastern hemlock mortality in a rural and an urban setting in New England,

Templer and McCann (2010) found that the sites responded to atmospheric N inputs differently. The urban forest had higher inputs and exports of N, but the N lost had an isotopic signature suggesting that vegetation and the microbial community transformed atmospheric N via nitrification before it was leached from the soil profile. Nitrate lost from their rural site had an isotopic signature similar to precipitation, indicating most N did not undergo biological transformation. Although less N was leached in the rural site overall, slow vegetation establishment and/or weak demand for N may have limited the capacity of biotic sinks to keep pace with N inputs (Templer and McCann 2010).

Tree growth data suggest that biotic demand was strong enough to decrease subsurface N flux at high elevations but not at low elevations. One possible explanation for this pattern is that stands are co-limited by phosphorus (P) and hemlock mortality enhanced P availability in high, but not low or mid-elevation stands. In a companion study, Block and others (in press) found higher fluxes of dissolved reactive P and increased P in foliage and forest floor wood in declining hemlock stands at high elevations (with high N deposition). If hemlock mortality increases P availability, there may be an increased capacity for N uptake due to stimulation of primary production. This agrees with the temporal increase in BAI of *Betula alleghaniensis* and *Acer pensylvanicum* in high elevation hemlock stands post-infestation.

Previous studies have suggested increases in biotic N pools may indicate symptoms of N excess in ecosystems (Aber and others 1998; Fenn and others 1998). However, Lovett and Goodale (2011) recently suggested that N can flow to sinks and be lost from a system simultaneously, with the fate and temporal patterns of N flow dependent on the strength of the sinks and the factors that control them. For example, exogenous N may be transferred to biotic pools such as foliage, stored in wood, and allocated for increased tree growth, even as some losses occur. Interpreted in this light, our results suggest that, although high elevation stands may be progressing toward N saturation, soil and vegetation sinks still have sufficient capacity to store exogenous N. However, the observed differences in N fluxes with temporal period demonstrate that sink strength can vary with other factors.

CONCLUSIONS

Our results suggest that the impacts of HWA will be manifested in different ways across the various ecosystems found within the native range of

hemlock. Eastern hemlock is a sub-dominant species in southern Appalachians forests and will likely liberate less N compared to pure hemlock stands further north. Additionally, our findings suggest a paradox that hemlock decline has enhanced N retention under conditions of high N availability by increasing biotic N demand in hardwoods that co-occur in these mixed stands. The impacts of pest-induced tree decline on N retention may thus depend on the capacity of compensating biotic sinks to capture and sequester available N. Given that exotic insects and pathogens are now a widespread, persistent problem in many forests, understanding the controls on sink strengths is an imperative, yet complex challenge.

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REFERENCES

Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems—Hypotheses revisited. Bioscience 48:921–34.

Aber JD, Goodale CL, Ollinger SV, Smith ML, Magill AH, Martin ME, Hallett RA, Stoddard JL. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? Bioscience 53:375–89.

Block CE, Knoepp JD, Fraterrigo JM. Interactive effects of disturbance and nitrogen availability on phosphorus dynamics of Southern Appalachian forests. Biogeochemistry (in press).

Boettcher SE, Kalisz PJ. 1990. Single-tree influence on soil properties in the mountains of eastern Kentucky. Ecology 71:1365–72.

Day FP, Phillips DL, Monk CD. 1988. Forest communities and patterns. In: Swank WT, Crossley DA Jr, Eds. Forest hydrology and ecology at Coweeta. New York: Springer-Verlag. p 141–9.

Elliott KJ, Vose JM. 2011. The contribution of the Coweeta Hydrologic Laboratory to developing an understanding of long-term (1934–2008) changes in managed and unmanaged forests. For Ecol Manage 261:900–10.

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Front Ecol Environ 3: 479–86.

Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan DE, Stottlemyer R. 1998. Nitrogen excess in North American ecosystems: predisposing factors, ecosystem responses, and management strategies. Ecol Appl 8:706–33.

Ford CR, Vose JM. 2007. *Tsuga canadensis* (L.) Carr. mortality will impact hydrologic processes in southern Appalachian forest ecosystems. Ecol Appl 17:1156–67.

Ford C, Elliott K, Clinton B, Kloeppel B, Vose J. 2011. Forest dynamics following eastern hemlock mortality in the southern Appalachians. Oikos 121:523–536.

Fritts HC. 1972. Tree rings and climate. Sci Am 226:92–100.

Gandhi KJK, Herms DA. 2010. Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biol Invasions 12:389–405.

Jenkins JC, Aber JD, Canham CD. 1999. Hemlock woolly adelgid impacts on community structure and N cycling rates in eastern hemlock forests. Can J For Res 29:630–45.

Kizlinski ML, Orwig DA, Cobb RC, Foster DR. 2002. Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. J Biogeogr 29:1489–503.

Knoepp JD, Vose JM, Swank WT. 2008. Nitrogen deposition and cycling across an elevation and vegetation gradient in southern Appalachian forests. Int J Environ Stud 65:389–408.

Knoepp JD, Vose JM, Clinton BD, Hunter MD. 2011. Hemlock infestation and mortality: impacts on nutrient pools and cycling in Appalachian forests. Soil Sci Soc Am J 75:1935–45.

Langlois JL, Johnson DW, Mehuys GR. 2003. Adsorption and recovery of dissolved organic phosphorus and nitrogen by mixed-bed ion-exchange resin. Soil Sci Soc Am J 67:889–94.

Levine JM, D'Antonio CM. 2003. Forecasting biological invasions with increasing international trade. Conserv Biol 17:322–6.

Lovett GM, Goodale CL. 2011. A new conceptual model of nitrogen saturation based on experimental nitrogen addition to an oak forest. Ecosystems 14:615–31.

Lovett GM, Bowser JJ, Edgerton ES. 1997. Atmospheric deposition to watersheds in complex terrain. Hydrol Process 11:645–54.

Lovett GM, Weathers KC, Arthur MA, Schultz JC. 2004. Nitrogen cycling in a northern hardwood forest: do species matter? Biogeochemistry 67:289–308.

Lovett GM, Canham CD, Arthur MA, Weathers KC, Fitzhugh RD. 2006. Forest ecosystem responses to exotic pests and pathogens in eastern North America. Bioscience 56:395–405.

McClure MS. 1991. Density-dependent feedback and population-cycles in *Adelges tsugae* (Homoptera, Adelgidae) on *Tsuga canadensis*. Environ Entomol 20:258–64.

Nuckolls A, Wurzburger N, Ford C, Hendrick R, Vose J, Kloeppel B. 2009. Hemlock declines rapidly with hemlock woolly adelgid infestation: impacts on the carbon cycle of Southern Appalachian Forests. Ecosystems 12:179–90.

Ollinger SV, Smith ML, Martin ME, Hallett RA, Goodale CL, Aber JD. 2002. Regional variation in foliar chemistry and N cycling among forests of diverse history and composition. Ecology 83:339–55.

Orwig DA, Cobb RC, D'Amato AW, Kizlinski ML, Foster DR. 2008. Multi-year ecosystem response to hemlock woolly adelgid infestation in southern New England forests. Can J For Res 38:834–43.

Qualls RG, Haines BL, Swank WT, Tyler SW. 2000. Soluble organic and inorganic nutrient fluxes in clearcut and mature deciduous forests. Soil Sci Soc Am J 64:1068–77.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. 2nd edn. Cambridge: Cambridge University Press.

Stadler B, Muller T, Orwig D, Cobb R. 2005a. Hemlock woolly adelgid in New England forests: canopy impacts transforming ecosystem processes and landscapes. Ecosystems 8:233–47.

Stadler B, Müller T, Orwig D, Cobb R. 2005b. Hemlock woolly adelgid in New England forests: canopy impacts transforming ecosystem processes and landscapes. Ecosystems 8:233–47.

Susfalk RB, Johnson DW. 2002. Ion exchange resin based soil solution lysimeters and snowmelt solution collectors. Commun Soil Sci Plant Anal 33:1261–75.

Templer PH, McCann TM. 2010. Effects of the Hemlock woolly adelgid on nitrogen losses from urban and rural northern forest ecosystems. Ecosystems 13:1215–26.

Templer PH, Lovett GM, Weathers KC, Findlay SE, Dawson TE. 2005. Influence of tree species on forest nitrogen retention in the Catskill Mountains, New York, USA. Ecosystems 8:1–16.

van Breemen N, Boyer EW, Goodale CL, Jaworski NA, Paustian K, Seitzinger SP, Lajtha K, Mayer B, van Dam D, Howarth RW, Nadelhoffer KJ, Eve M, Billen G. 2002. Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry 57–58:267–93.

Weathers KC, Lovett GM, Likens GE, Lathrop R. 2000. The effect of landscape features on deposition to Hunter Mountain, Catskill Mountains, New York. Ecol Appl 10:528–40.

Weathers KC, Simkin SM, Lovett GM, Lindberg SE. 2006. Empirical modeling of atmospheric deposition in mountainous landscapes. Ecol Appl 16:1590–607.

Yorks TE, Leopold DJ, Raynal DJ. 2003. Effects of *Tsuga canadensis* mortality on soil water chemistry and understory vegetation: possible consequences of an invasive insect herbivore. Can J For Res 33:1525–37.