Effects of the Hemlock Woolly Adelgid on Nitrogen Losses from Urban and Rural Northern Forest Ecosystems

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ABSTRACT

The objectives of this study were to quantify rates of nitrogen inputs to the forest floor, determine rates of nitrogen losses via leaching and to partition the sources of NO_3^- from healthy, declining, and salvage or preemptively cut eastern hemlock (*Tsuga canadensis*) stands in both an urban forest at the Arnold Arboretum in Boston, MA and a rural forest at Harvard Forest in Petersham, MA. Rates of nitrogen inputs (NH_4^+ and NO_3^-) to the forest floor were 4–5 times greater, and rates of nitrogen losses via leachate were more than ten times greater, at the Arnold Arboretum compared to Harvard Forest. Nitrate that was lost via leachate at Harvard Forest came predominantly from atmospheric deposition inputs, whereas NO_3^- losses at the Arnold Arboretum came predominantly from nitrification. Although our study was limited to one urban and one rural site, our results suggest that current management regimes used to control the hemlock woolly adelgid (*Adelges tsugae*), such as salvage cutting, may be reducing nitrogen losses in urban areas due to rapid regrowth of vegetation and uptake of nitrogen by those plants. In contrast, preemptive cutting of trees in rural areas may be leading to proportionately greater losses of nitrogen in those sites, though the total magnitude of nitrogen lost is still smaller than in urban sites. Results of our study suggest that the combination of the hemlock woolly adelgid, nitrogen inputs, and management practices lead to changes in the movement and source of NO_3^- losses from eastern hemlock forest ecosystems.

Key words: forest insect outbreak; invasive species; natural abundance stable isotopes; nitrate sources; nitrogen saturation; pest management; *Tsuga canadensis*.

INTRODUCTION

Human-induced environmental changes, such as the introduction of non-native species, have led to dramatic shifts in forest composition in the eastern United States over the past 100 years. Introduced pest species, such as the Asian longhorned beetle (*Anoplophora glabripennis*), chestnut blight (*Cryphonectria parasitica*), Dutch elm disease (*Ophiostoma* spp.), the emerald ash borer (*Agrilus* spp.), the gypsy moth (*Lymantria dispar* L), and the hemlock woolly adelgid (*Adelges tsugae* Annand), have led to the decline of native tree species throughout this region. The hemlock woolly adelgid, an introduced

aphid-like insect from Japan, threatens hemlock stands throughout the eastern United States. The hemlock woolly adelgid was first reported in forests of the eastern United States in the early 1950s and is currently leading to mortality of eastern hemlock trees from Georgia to Massachusetts (McClure and Cheah 1999; Orwig and others 2002).

The hemlock woolly adelgid suffers significant mortality at winter temperatures below $-25°C$ (Skinner and others 2003). With the combination of increasing temperatures and potentially greater cold tolerance of the hemlock woolly adelgid, stands of eastern hemlock trees could be affected by this pest throughout the entire range of this tree species within the next 30 years (Albani and others 2010). Feeding, and possible injection of toxic saliva, by the hemlock woolly adelgid on young branches and twigs reduces the viability of new growth and typically leads to tree mortality within 4–10 years (McClure 1987; Young and others 1995). Eastern hemlock trees are recognized as a foundation species because of the large effect they have on forest ecosystems that they inhabit (Ellison and others 2005). In healthy eastern hemlock stands, they produce a shady understory with a thick organic soil layer due to the poor quality (for example, low %N; Lovett and others 2004), acidic composition of their litter and because of slow rates of decomposition (McClaugherty and others 1985). As a result, they tend to be associated with relatively slow rates of nitrogen cycling (Finzi and others 1998; Jenkins and others 1999; Templer and others 2003; Lovett and others 2004) and relatively high amounts of ecosystem nitrogen retention (Templer and others 2005).

Mortality of eastern hemlock trees impacts nutrient cycling and nitrogen retention of forest ecosystems significantly. For example, canopy throughfall beneath infested hemlock trees has greater concentrations of NO_3^- (Stadler and others 2005) and soil solution within stands of girdled hemlock trees has elevated concentrations of NH_4^+ and NO_3^- (Yorks and others 2003). Girdling is a treatment used to mimic the decline of trees caused by the hemlock woolly adelgid. Trees typically remain standing following both girdling and infestation by the hemlock woolly adelgid, but trees can remaining standing longer when infested with the hemlock woolly adelgid. Changes in the microclimate caused by openings in the canopy from declining hemlock stands lead to increased rates of net mineralization, net nitrification, and nitrogen turnover in the forest floor (Jenkins and others 1999; Orwig and others 2008). Mixed hardwood stands dominated by black birch (*Betula lenta*)

typically replace hemlock stands following the decline of hemlock trees (Orwig and Foster 1998; Albani and others 2010). This shift in tree species composition leads to increased rates of litter decomposition, which will likely influence long-term changes in nitrogen cycling (Cobb 2010).

In response to hemlock woolly adelgid outbreaks, many hemlock stands have been cut to salvage usable wood before tree mortality (Orwig and others 2002). However, silvicultural management of infested hemlock stands may also play a significant role in altering nitrogen cycling at those sites where trees are cut (Foster and Orwig 2006). For example, logging of hemlock forests leads to greater rates of net nitrification and standing pools of inorganic nitrogen compared to both declining and healthy hemlock stands (Kizlinski and others 2002). In fact, pre-emptive cutting of hemlock stands can cause greater losses of NO_3^- via leaching compared to allowing the slow decline of hemlock woolly adelgid-infested stands (Kizlinski and others 2002).

Although several studies have shown that mortality and management of eastern hemlock trees both lead to greater rates of NO_3^- losses compared to relatively healthy hemlock stands, it is not known what proportion of leached NO_3^- comes directly from atmospheric deposition and canopy throughfall versus nitrification. It is possible that during the declining phase of eastern hemlock forests (before they are replaced by healthy hardwood forests), both plant and microbial demand for nitrogen could be reduced and therefore a greater proportion of nitrogen could be lost from the forest due to a temporary 'saturation' of biotic demand. In this case, the predominant source of NO_3^- leached from declining stands would be directly from atmospheric deposition (and not biologically processed via nitrification). Alternatively, microbial processes such as nitrification, which has been documented to increase following hemlock forest decline (Jenkins and others 1999; Orwig and others 2008), could account for a large proportion of NO_3^- losses.

Natural abundance $\delta^{15}N$ values of NO_3^- have been used in ecosystem studies to partition sources of nitrogen, such as coal-fired power plants versus tailpipe exhaust, because these sources of nitrogen vary in their $\delta^{15}N$ isotopic signatures (Moore 1977; Heaton 1990; Elliott and others 2007). However, natural abundance $\delta^{15}N$ values alone cannot be used to partition sources of NO_3^- between atmospheric inputs and biological processes such as nitrification because of the overlapping isotopic composition of atmospherically derived and mi-

crobially produced $^{15}NO_3^-$ (Kendall and McDonnell 1998). In contrast, natural abundance $\delta^{18}O$ values of NO_3^- differ significantly between NO_3^- of precipitation and NO_3^- produced microbially during the process of nitrification. Therefore, the $\delta^{18}O$ signature of NO_3^- in soil solution or stream water can be measured and, with a two end-member mixing model, the predominant source of NO_3^- being leached from a terrestrial ecosystem can be determined. Numerous studies have employed $\delta^{18}O$ values for partitioning NO_3^- sources in terrestrial ecosystems (Böttcher and others 1990; Durka and others 1994; Kendall and others 1996; Willard and others 2001; Burns and Kendall 2002; Campbell and others 2002; Ohte and others 2004; Pardo and others 2004). These studies have shown that with the exception of declining forest stands that receive exceptionally high rates of nitrogen inputs via deposition (for example, Durka and others 1994), most forest ecosystems lose NO_3^- that comes predominantly from nitrification, rather than directly from precipitation.

The objectives of this study were to quantify rates of nitrogen inputs to the forest floor, determine rates of nitrogen losses via leaching and to partition the sources of NO_3^- from healthy, declining and cut eastern hemlock stands. As part of this objective, we examined hemlock stands within an urban site in Boston, MA (Arnold Arboretum), as well as hemlock and hardwood stands at a rural site in Petersham, MA (Harvard Forest). We expected that rates of both nitrogen inputs and outputs would be greater at the Arnold Arboretum compared to Harvard Forest. We also predicted that declining and cut hemlock stands at both sites would show a greater proportion of NO_3^- in leachate directly from atmospheric inputs due to saturation of biotic demand for nitrogen, whereas healthy stands would have a greater proportion of NO_3^- lost primarily from nitrification.

SITE DESCRIPTIONS

Sampling for the rural site occurred at the Simes tract at the Harvard Forest Long-Term Ecological Research site in Petersham, MA. This area was cleared for agricultural use and began to regenerate as forest in the late 1800s and early 1900s (Kernan 1980). Soils at this site are acidic with a bulk density equal to 0.15 g cm^{-3} and 0.34 g cm^{-3} in the organic and mineral horizons, respectively (pH = 3.12 organic horizon and 3.81 in mineral horizon; K Savage and D Orwig, *personal communication*). The soils are predominantly coarse-loamy, mixed, active, mesic Typic Dystrudepts in the

Charlton Series that are derived from glacial till. Eight 90×90 m^2 plots were established in 2003 and experimental treatments were begun in 2005. Treatments were randomly assigned to the plots. Eastern hemlock trees in two of the plots were girdled in May 2005 to simulate hemlock woolly adelgid induced decline of eastern hemlock trees. All girdled trees were left standing, but died within 2 years of the treatment, which is relatively shorter than the amount of time most eastern hemlock trees die from the adelgid (Ellison and others 2010). Two plots were logged (''cut'') between February and April 2005 to mimic the effect of commercial logging of eastern hemlock forests. The slash was left on site in these plots. Two plots were left undisturbed (''control''). Two hardwood plots (''hardwood controls'') were used to mimic the projected tree species composition of forests following the decline of eastern hemlock trees (Orwig and Foster 1998; Albani and others 2010). Hardwood tree species in the two hardwood plots include *Acer rubrum* L. (red maple), *Quercus rubra* L. (red oak), *Q. alba* L. (white oak), and *Betula lenta* L. (black birch; Ellison and others 2010). Eastern hemlock and red maple trees dominate the central, poorly drained portion of this area. This research is part of a larger project examining the impact of the hemlock woolly adelgid on a variety of forest ecosystem properties, including air and soil temperature, rates of nutrient cycling, carbon fluxes and plant and animal species composition (Ellison and others 2010).

The nearest National Atmospheric Deposition (NADP) Network National Trends Network (NTN) site to the Harvard Forest study site is located approximately 26 km away at the Quabbin Reservoir (MA 08; 42.3925 north latitude and -72.3444 longitude). Mean annual rates of bulk deposition between 2000 and 2008 for the Quabbin Reservoir were 1.88 kg NH$_4$-N ha^{-1} y^{-1} and 3.11 kg NO$_3$-N ha^{-1} y^{-1} (NADP 2010). Estimates for Harvard Forest for wet and dry deposition from regional extrapolation of NADP–NTN data are 5.1 and 2.5 kg N ha^{-1} y^{-1}, respectively (Ollinger and others 1993). Estimates from eddy covariance measurements for total deposition are 6.6 kg N ha^{-1} y^{-1} (Munger and others 1996).

To understand the impact of the hemlock woolly adelgid and changes in forest structure on ecosystem processes in an urban forest, sampling was also conducted at the Hemlock Hill tract of the Arnold Arboretum in Boston, MA. The hemlock woolly adelgid was discovered at the Arnold Arboretum in 1997; most of the hemlock trees at this site are currently infested or have already died from the

hemlock woolly adelgid. Working at this urban site allowed us to examine a mature second-growth stand currently being affected by the hemlock woolly adelgid and to examine these stands in an urban context, with likely greater rates of nitrogen inputs via deposition (Schultz 1994) and higher temperatures due to the heat island effect, compared to the rural site at Harvard Forest. Because colder temperatures are thought to limit the northern extent of the range of the hemlock woolly adelgid (Skinner and others 2003), urban areas may potentially extend this pest's range north with their warmer temperatures than surrounding areas. Soils at our plots at the Arnold Arboretum are acidic with a bulk density equal to 0.39 and 1.15 g cm^{-3} in the organic and mineral horizons, respectively (pH = 2.95 organic horizon and 3.91 in mineral horizon; Orwig and Foster 2009). Six 15 × 15 m^2 plots were established at Hemlock Hill at the Arnold Arboretum in 2004. Hemlock trees were removed via cutting from four of the six plots in early 2005 to examine the impact of salvage logging on ecosystem processes. The slash created from cutting was chipped on site and left in two of the plots ("chipped") and was removed from the other two plots ("cut") to evaluate post-cut impacts on forest processes. Two plots were left undisturbed ("control").

The nearest NADP–NTN site to the Arnold Arboretum study site is located approximately 16 km away in Middlesex County, MA (MA 13; 42.3839 north latitude and −71.2147 longitude). Mean annual rates of bulk deposition between 2000 and 2008 for Middlesex County were 1.51 kg NH$_4$-N ha^{-1} y^{-1} and 2.51 kg NO$_3$-N ha^{-1} y^{-1} (NADP 2010). Estimates for the Arnold Arboretum for wet and dry deposition from regional extrapolation of NADP–NTN data are 4.1 and 2.6 kg N ha^{-1} y^{-1}, respectively (Ollinger and others 1993).

METHODS

We used throughfall collectors to measure nitrogen inputs to the forest floor. Within each of the 14 plots ($n = 8$ at Harvard Forest and $n = 6$ at the Arnold Arboretum), we placed two 20 ml disposable chromatography columns (0.25″ diameter), each attached to the top of a 3.5 m PVC pipe (buried to 0.5 m depth). The columns were packed with Dowex Monosphere MR-3 UPW mixed ion exchange resin. A 30 μm pore-size filter was placed at the bottom of each resin column. A 20 cm diameter funnel was placed on top of the column to collect rain water and canopy throughfall. Poly wool was placed in the funnel to prevent solid

debris from entering the resin column. Some of the nitrogen that accumulated on the resin beads could have come from insect frass that was deposited onto the funnels and decomposed within the period the resin columns were left in the field. Resin columns were replaced with a new collector every 6 weeks between May and November 2006. Although an annual measurement of deposition would be ideal, for this project we measured bulk deposition and canopy inputs throughout the snow-free months of the year only.

Four ion exchange resin bags were placed in the mineral soil of each of the 14 plots during the growing and dormant seasons to provide a relative index of nitrogen leaching (Giblin and others 1994). Within each plot, two resin bags were paired with each of the two resin columns (approximately 1 m from the bottom of each PVC pipe in opposite cardinal directions). Bags were placed in the soil by inserting a flat pry bar at an angle to create a slit extending into the mineral soil. The resin bags were placed in this slit to a depth of greater than 10 cm, which was below the zone of high fine root density. Each resin bag was packed with 10 g of Dowex Monosphere MR-3 UPW mixed Ion Exchange Resin. The first set of resin bags was inserted in mineral soils in May 2006, the first full year following treatment, and was replaced once with new bags mid-way through the 2006 growing season. A third set remained in the plots throughout the 2006/2007 dormant season (September 2006–May 2007).

We extracted both the resin columns and resin bags with 2 M KCl. Fifty milliliters of 2 M KCl was added three times sequentially (150 ml total) to resin columns and resin bags, filtered through a Whatman #1 filter and a sub-sample of solution was analyzed on a Lachat QuikChem 8500 flow-injection analyzer. We used the salicylate method (E10-107-0602-A) for NH$_4^+$ concentrations in solution and the nitrate/nitrite—8000 method (E10-107-04-1-C) for determining NO$_3^-$ + NO$_2^-$ (hereafter NO$_3^-$) concentrations in solution. The external calibration standards used were NH$_4^+$ (Environmental Resource Associates catalog #985) and NO$_3^-$ (ERA catalog #991). To determine the total amount of nitrogen inputs throughout the growing season (May–November), the amount of nitrogen in the resin columns was summed for each location within each plot over time. To quantify the mass of nitrogen in soil leachate during the growing season, we summed the amount of nitrogen extracted from resin bags over the two sampling periods (May–June and June–September 2006) for each location within each plot. For the

dormant season, we calculated the total amount of nitrogen extracted from the resin bags left in the field September 2006–May 2007. Values for each pair of resin bags (leachate) were averaged prior to statistical analysis. Values for resin columns were averaged within each plot prior to analysis.

We determined $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- extracted from the mixed resin using the denitrifier method (Casciotti and others 2002), which provides the determination of $\delta^{15}N$ values in low N concentration samples (that is, atmospheric deposition and stream samples), as well as the $\delta^{18}O$ of NO_3^-, which is not possible using the traditional ^{15}N diffusion method. We incubated 1 ml of KCl extract solutions containing NO_3^- overnight in 20 ml test tubes containing denitrifying bacteria (*Pseudomonas aureofaciens*) that lack active N_2O reductase. Before injecting the samples into the test tubes, the vials containing bacteria were crimp sealed, flushed with N_2 for 3 h and antifoam B was added. Following 24 h of incubation, 5 drops (approximately 0.5 ml) of 12 M NaOH were added to stop the reaction and to kill the bacteria. $\delta^{15}N_2^{18}O$ gas produced by the bacteria was measured on a SerCon Cryoprep trace gas concentration system interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (SerCon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility. Standard solutions (USGS standard #32, 34 and 35 = KNO_3, KNO_3, and $NaNO_3$, respectively) were processed with the same method as internal standards. Samples were run in increasing mass of nitrogen to correct for mass based isotope effects within each batch. Ten percent of samples were run in duplicate, providing us with an estimate of precision (0.015‰ for $\delta^{15}N$ and 0.0093‰ for $\delta^{18}O$; standard error). We used isotopic values for samples whose beam areas (Beam 44) on the isotope ratio mass spectrometer were at least ten times greater than the beam area of our blanks. The minimum detection limit for this method was approximately 10 nmol of nitrogen.

Nitrogen isotopic values are expressed as $\delta^{15}N$ values where the standard is atmospheric air, which is defined as 0‰. Oxygen isotopic values are expressed as $\delta^{18}O$ values where the standard is Vienna standard mean ocean water, also defined as 0‰.

$$\delta^{15}N(\text{‰}) = \left(\frac{(^{15}N/^{14}N\,\text{sample})}{(^{15}N/^{14}N\,\text{standard})} - 1 \right) \times 1000 \quad (1)$$

$$\delta^{18}O(\text{‰}) = \left(\frac{(^{18}O/^{16}O\,\text{sample})}{(^{18}O/^{16}O\,\text{standard})} - 1 \right) \times 1000 \quad (2)$$

We used the following mixing model equation to partition sources of NO_3^- in leachate between atmospheric inputs and nitrification,

% Contribution from Atmospheric Inputs =

$$\left(\frac{\delta^{18}O_{\text{loss}} - \delta^{18}O_{\text{nitrifiaction}}}{\delta^{18}O_{\text{atm}} - \delta^{18}O_{\text{nitrifiaction}}} \right) \times 100$$

where $\delta^{18}O_{\text{atm}}$ is the value for $\delta^{18}O$ in atmospheric inputs and $\delta^{18}O_{\text{loss}}$ is the value for $\delta^{18}O$ in soil leachate.

Soils for microbial biomass nitrogen were collected in June 2006. The fumigation-extraction method was used to determine soil microbial biomass nitrogen (Vance and others 1987). Ten grams of each fresh organic and mineral soil sample ($n = 4$ per plot in each soil horizon) were fumigated with chloroform for 24 h to kill and lyse microbial cells in the sample. Both the fumigated and non-fumigated soil samples were extracted with 40 ml 0.5 M K_2SO_4, shaken for 1 h at 125 rpm and filtered through a Whatman #1 filter. Total dissolved nitrogen was determined for fumigated and non-fumigated soils by digesting 2 ml soil extract sample with 4 ml persulfate (Cabrera and Beare 1993). Samples were corrected for both mass loss and reagent blanks. Microbial biomass nitrogen was calculated as the difference in nitrogen mass between the fumigated and non-fumigated soils (Vance and others 1987).

STATISTICAL ANALYSES

We conducted an Analysis of Variance (ANOVA) using treatment (control, cut, girdled, and hardwood control at Harvard Forest and control, cut, and chipped at the Arnold Arboretum) within each site (Harvard Forest and Arnold Arboretum) as the main effect. We examined nitrogen inputs and losses as leachate within each season as the response variables. We conducted repeated measures ANOVA using treatment within each site as the main effect and $\delta^{15}N$ and $\delta^{18}O$ values in NO_3^- inputs and leachate as the response variables. We conducted a two-way ANOVA using treatment and soil depth as the main effects within each site and examining soil microbial biomass nitrogen as the response variable. We also conducted an ANOVA comparing the control plots at each of the two sites for each response variable. We used the Shapiro–Wilk test to determine whether data were normally distributed. Data that were not normally distributed were log-transformed prior to statistical analysis. We tested for equal variance using Bartlett's

Test. We used Tukey's test for post hoc comparisons of means. We used SAS JMP software (Version 8.0.2, 2009) for all statistical analyses. We report means with standard error throughout the ''Results'' section.

RESULTS

Nitrogen Inputs and Losses

Rates of nitrogen inputs measured in this study for both NH_4^+ and NO_3^- at the Arnold Arboretum (9.6 ± 1.0 kg NH_4-N ha^{-1} y^{-1} and 5.3 ± 0.7 kg NO_3-N ha^{-1} y^{-1}, respectively, in control plots at the Arnold Arboretum; Figure 1) were greater than values reported by the NADP–NTN in Middelsex County, approximately 16 km away. We measured nitrogen inputs in the control plots at the Harvard Forest study site to be 2.5 ± 0.6 kg NH_4-N ha^{-1} y^{-1} and 1.1 ± 0.4 NO_3-N ha^{-1} y^{-1} (Figure 1). Nitrogen inputs to the forest floor as NH_4^+ and NO_3^- were four and five times, respectively, greater within the control plots at the Arnold Arboretum compared to the control plots at Harvard Forest ($P = 0.026$ and $P = 0.032$ for NH_4^+ and NO_3^-,

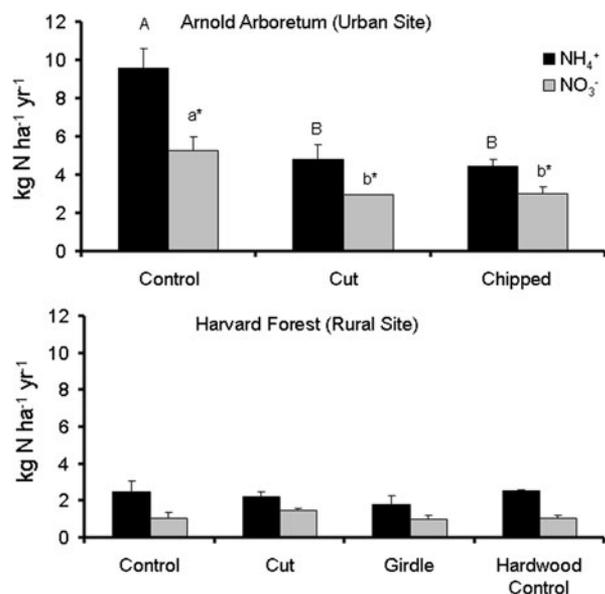
Figure 1. Nitrogen inputs as NH_4^+ and NO_3^- from atmospheric deposition and canopy throughfall at the Arnold Arboretum and Harvard Forest during the 2006 growing season. Values are means with one standard error ($n = 2$). *Different uppercase letters* represent statistically significant differences among treatments in NH_4^+ inputs within each site. *Different lowercase letters* represent statistically significant differences among treatments in NO_3^- inputs within each site. *$P = 0.057$.

respectively; Figure 1). At the Arnold Arboretum, both NH_4^+ and NO_3^- inputs to the forest floor were greater within the control plots compared to the cut and chipped plots ($P = 0.030$ and $P = 0.057$ for NH_4^+ and NO_3^-, respectively). At the Harvard Forest plots, treatment did not have a significant effect on either NH_4^+ or NO_3^- inputs ($P = 0.60$ and $P = 0.44$, respectively).

During the growing season, the amounts of NH_4^+ and NO_3^- in leachate were 14 and 30 times, respectively, greater in the control plots at the Arnold Arboretum compared to Harvard Forest ($P = 0.0030$ and $P = 0.0012$ for NH_4^+ and NO_3^-, respectively; Figure 2). At the Arnold Arboretum, there was 13 and 6 times as much NH_4^+ in the control plots compared to the chipped and cut plots, respectively ($P = 0.020$); there was six times as much NO_3^- in leachate within the control plots compared to the chipped plots ($P = 0.032$). At Harvard Forest, there was three times as much NH_4^+ in leachate in the cut plots compared to the hardwood control plots ($P = 0.034$). The amount of NH_4^+ in leachate in the control plots was smaller than the cut plots and greater than the girdled and hardwood control plots, but the difference was not statistically significant. There was no effect of treatment on NO_3^- in leachate in the growing season at Harvard Forest ($P = 0.66$).

During the winter dormant season, the amounts of NH_4^+ and NO_3^- in leachate were between 12 and 19 times greater in the control plots of the Arnold Arboretum compared to Harvard Forest ($P = 0.030$ and $P = 0.042$ for NH_4^+ and NO_3^- in the winter dormant season, respectively). The amount of NO_3^- in leachate was greater in the control plots compared to the cut and chipped plots at the Arnold Arboretum ($P = 0.066$) and was greater in the hardwood control plots compared to the girdled plots at Harvard Forest ($P = 0.034$). There were no treatment effects on NH_4^+ losses in winter at the Arnold Arboretum or Harvard Forest ($P = 0.24$ and $P = 0.92$, respectively).

Soil microbial biomass nitrogen was not significantly influenced by treatment within either site (Figure 3; $P = 0.36$ at the Arnold Arboretum and $P = 0.68$ at Harvard Forest). When expressed per mass soil, microbial biomass nitrogen was 1.6 times greater in the control plots at Harvard Forest compared to the control plots at Arnold Arboretum ($P = 0.026$; Figure 3). In contrast, when expressed per unit area, microbial biomass nitrogen was 1.7 times greater in the control plots at the Arnold Arboretum compared to Harvard Forest ($P = 0.015$). At both sites, microbial biomass nitrogen per unit soil and per unit area were significantly greater in
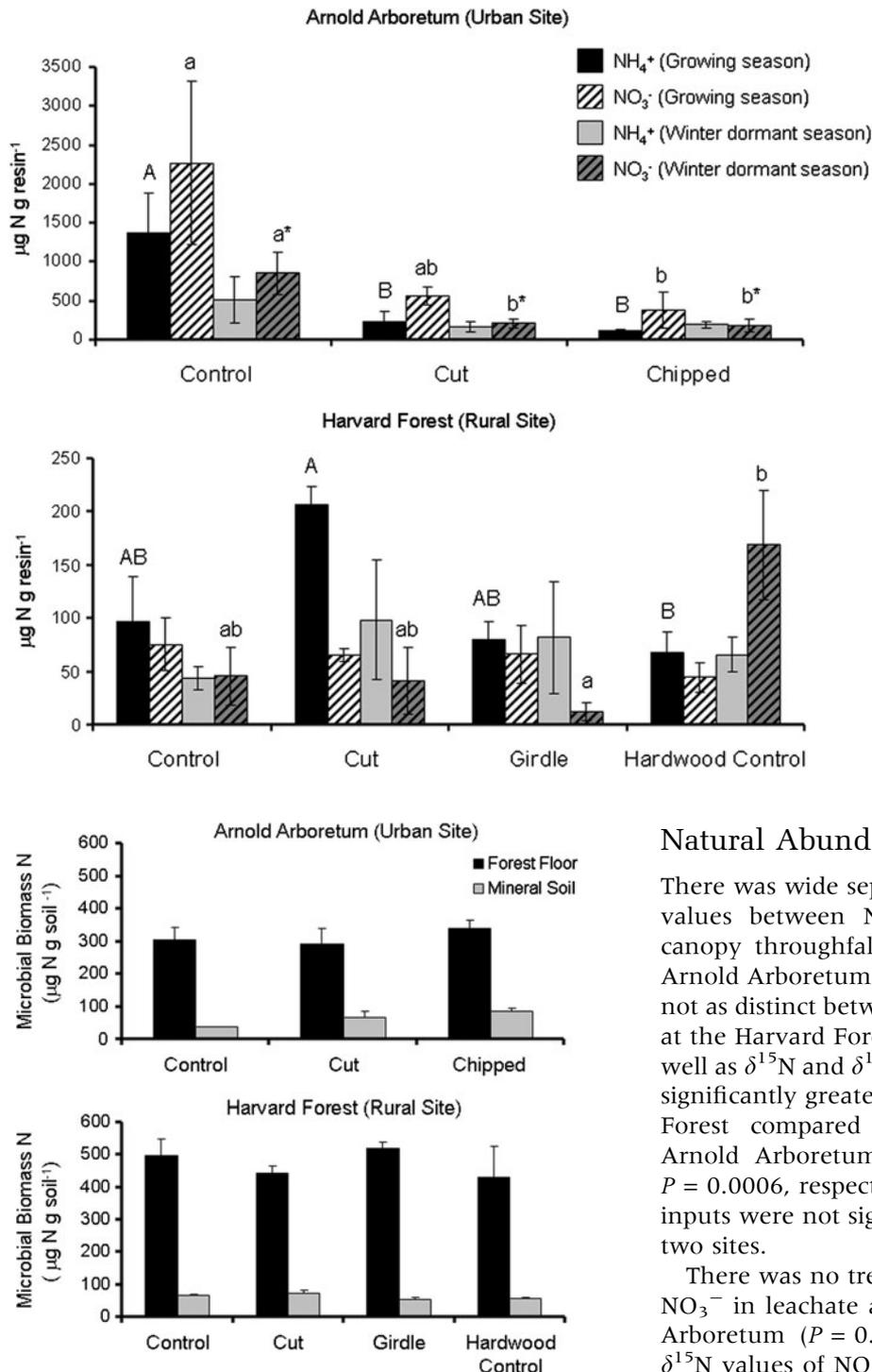
Figure 2. Nitrogen in soil leachate as NH_4^+ and NO_3^- during the 2006 growing season (May–September 2006) and the 2006–2007 winter dormant season at the Arnold Arboretum and Harvard Forest. Values are means with one standard error ($n = 4$). *Different uppercase letters* represent statistically significant differences among treatments in leachate NH_4^+ within each site and season. *Different lowercase letters* represent statistically significant differences among treatments in leachate NO_3^- within each site and season. $*P = 0.066$.

Figure 3. Microbial biomass nitrogen within the forest floor and mineral soil at the Arnold Arboretum and Harvard Forest. Data are means with one standard error ($n = 2$).

the forest floor compared to the mineral soil ($P = 0.0004$ and $P = 0.0015$ per mass soil and per unit area, respectively).

Natural Abundance Isotope Values

There was wide separation in mean $\delta^{15}N$ and $\delta^{18}O$ values between NO_3^- inputs in deposition and canopy throughfall and NO_3^- in leachate at the Arnold Arboretum (Figure 4). Isotopic values were not as distinct between inputs and outputs of NO_3^- at the Harvard Forest. The $\delta^{18}O$ of NO_3^- inputs, as well as $\delta^{15}N$ and $\delta^{18}O$ for NO_3^- in leachate were all significantly greater in the control plots at Harvard Forest compared to the control plots at the Arnold Arboretum ($P = 0.0029$, $P = 0.0024$, and $P = 0.0006$, respectively). The ^{15}N values for NO_3^- inputs were not significantly different between the two sites.

There was no treatment effect on $\delta^{18}O$ values in NO_3^- in leachate at Harvard Forest or the Arnold Arboretum ($P = 0.36$ and $P = 0.75$, respectively). $\delta^{15}N$ values of NO_3^- inputs varied with treatment at both sites (Figure 4). $\delta^{15}N$ values in NO_3^- inputs were significantly greater within the control plots compared to the cut and chipped plots at the Arnold Arboretum ($P = 0.059$) and were greater within the control and hardwood control plots compared to the girdled and cut plots at Harvard Forest ($P = 0.01$). $\delta^{15}N$ values of NO_3^- in leachate were significantly greater within the hardwood control plots compared to the cut plots at Harvard
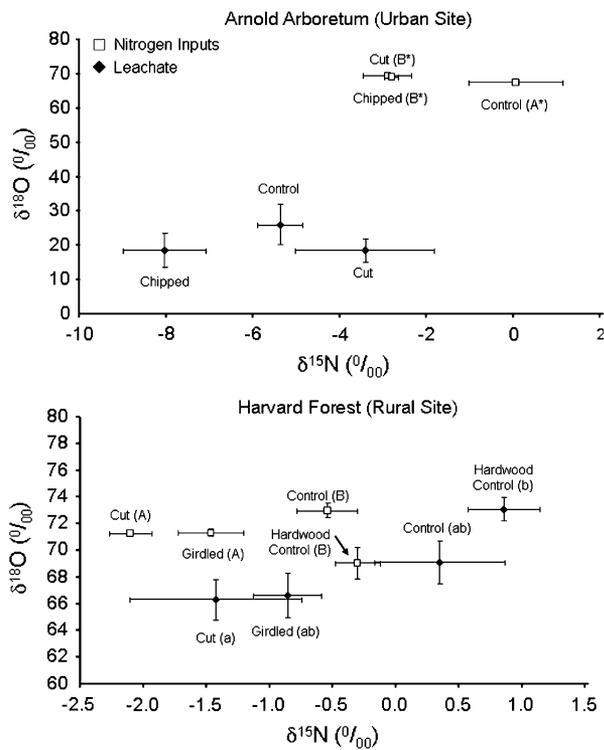
Figure 4. $\delta^{15}N$ and $\delta^{18}O$ values in NO_3^- inputs, from atmospheric deposition and canopy throughfall, and in soil leachate at the Arnold Arboretum and Harvard Forest. Data are means with one standard error for both $\delta^{15}N$ and $\delta^{18}O$ values. *Different uppercase letters* represent statistically significant differences among treatments in $\delta^{15}N$ of nitrogen inputs within each site. *Different lowercase letters* represent statistically significant differences among treatments in $\delta^{15}N$ values of leachate within each site. *$P = 0.059$. There were no significant differences among $\delta^{18}O$ values in nitrogen inputs or leachate.

Forest ($P = 0.037$). There was no impact of treatment on $\delta^{15}N$ values of leachate at the Arnold Arboretum ($P = 0.18$).

DISCUSSION

The goal of this study was to examine how the potential loss of a foundation species, eastern hemlock, could impact nutrient inputs to the forest floor, nitrogen losses via leaching and sources of NO_3^- in leachate in healthy, declining and cut eastern hemlock stands of an urban forest in Boston, MA and a rural forest in Petersham, MA. Determining the impact of mortality of eastern hemlock trees could enable better predictions about both the role of foundation species in nutrient cycling and how eastern forests could change with the loss of this tree species. As expected, rates of both nitrogen inputs and outputs were greater at

the urban site (Arnold Arboretum) compared to the rural site (Harvard Forest). Surprisingly, a larger proportion of NO_3^- in leachate came directly from atmospheric inputs at the Harvard Forest, whereas a larger proportion of NO_3^- in leachate came from nitrification at the Arnold Arboretum. Cutting led to greater nitrogen losses at the rural site, but led to reduced nitrogen losses at the urban site. We expected that hemlock mortality would lead to changes in nitrogen cycling within soils, which would impact the source (for example, direct deposition vs. nitrification) of NO_3^- in leachate. Contrary to our expectations, our results show that the decline of hemlock trees (via cutting, girdling, or infestation with the hemlock adelgid) had no impact on the source of NO_3^- lost from either site.

Nitrogen Inputs

Differences in canopy processes, methodology, and location may explain the higher rate of nitrogen inputs we found at the Arnold Arboretum compared to the NADP–NTN site in Middlesex County (~16 km away), and to regional extrapolations of NADP–NTN data (Ollinger and others 1993). The NADP–NTN program measures bulk deposition, which includes wet deposition inputs only, whereas we measured nitrogen inputs beneath the canopy, which includes both wet and dry deposition. Therefore, it is not surprising that rates of inputs were higher within all of our plots compared to those out in the open as measured by NADP–NTN. In support of this, the control plots at the Arnold Arboretum had greater nitrogen inputs compared to the cut and chipped plots (Figure 1), which is consistent with the larger canopy and greater interception of dry deposition in the control plots (Lovett 1994). The larger nitrogen inputs within the control plots, compared to the cut and chipped plots, could also be due to throughfall that was enriched in nitrogen from hemlock woolly adelgid-infested crowns, which have been found to contain significantly larger foliar microbe populations (Stadler and others 2005, 2006). Insect frass from the canopy in the control plots could have also contributed to the greater nitrogen inputs in these plots. An additional explanation is that our plots at the Arnold Arboretum are located in the city of Boston. In contrast, the NADP–NTN site in Middlesex County is located northwest of the city, which is upwind of the prevailing winds for this region and likely to have a relatively smaller amount of nitrogen in deposition (Schultz 1994). Rates of nitrogen deposition from regional extrapolations of NADP–NTN data (Ollinger and others

1993) are closer to our estimates, but not as high, which may be due potentially to local sources of nitrogen not measured by the NADP–NTN program or incorporated into regional models. To our knowledge, this is the first study documenting rates of nitrogen inputs within the city of Boston. Our observation that rates of nitrogen inputs were 4–5 times greater at the Arnold Arboretum compared to Harvard Forest is important for understanding nitrogen dynamics in each of these sites.

The difference between our measurements of nitrogen inputs at Harvard Forest and those reported by the NADP–NTN was smaller compared to the differences between our measurements and those reported by the NADP for the Arnold Arboretum. The relatively small difference in measured NH_4^+ inputs between our study site at Harvard Forest and those of the NADP is surprising considering that we measured canopy throughfall, whereas the NADP measures bulk deposition.

Nitrogen Losses

Similar to other studies comparing urban to rural areas, we observed significantly greater inputs and losses of nitrogen at the Arnold Arboretum compared to Harvard Forest. In addition to differences in proximity to urban areas, the control plots at each site differed in infestation by the hemlock woolly adelgid. At the time of this study, the control plots at the Arnold Arboretum were infested with the hemlock woolly adelgid, whereas the control plots at Harvard Forest were not. A study examining the impact of the hemlock woolly adelgid in southern Appalachian forests showed that infested canopies can have changes in carbon cycling process (for example, tree growth, belowground root production, and soil respiration) similar to girdling treatments (Nuckolls and others 2009). Therefore, it is not possible to determine whether the differences in control stands between the two sites are due to rural versus urban effects alone or due to the infestation of the hemlock woolly adelgid. However, all of the stands at the Arnold Arboretum had significantly greater amounts of nitrogen inputs and losses than stands at Harvard Forest, including the girdled plots at Harvard Forest, suggesting that proximity to an urban area had a significant impact on nitrogen inputs and losses.

Ecosystems with greater nitrogen inputs typically have greater nitrogen losses (Van Breeman and others 2002). However, we were surprised to observe greater nitrogen losses in leachate from the control plots compared to the cut or chipped plots

at the Arnold Arboretum because previous studies have shown that clearing results in temporary elevated losses of NO_3^- (Fisk and Fahey 1990). One possible mechanism for the smaller nitrogen losses from the cut and chipped plots at the Arnold Arboretum could be the rapid regrowth of vegetation following cutting in both of these plots, which began to occur within the first year following cutting (Lux 2009). In fact, regrowth of vegetation following cutting was five times faster at the Arnold Arboretum compared to Harvard Forest (Lux 2009), which could have been due in part to greater nitrogen inputs at the urban site compared to the rural site (Figure 1). With significant growth of herbaceous, shrub and tree species in the cut plots at the Arnold Arboretum, it is possible that there was greater plant uptake of nitrogen compared to the control plots, which were infested with the hemlock woolly adelgid. The combination of significant growth in the cut plots and diminished uptake in the infested, control plots could have resulted in smaller nitrogen losses in the cut plots compared to the control plots.

We also observed a treatment effect on nitrogen losses at Harvard Forest, with significantly greater losses of NH_4^+ in the cut plots compared to the hardwood control plots. Although vegetation grew in the cut plots at Harvard Forest following removal of hemlock trees, regrowth occurred at a relatively slower rate compared to the Arnold Arboretum (Lux 2009), perhaps explaining why new growth of vegetation did not take up and retain significant amounts of nitrogen in the cut plots. Although our work is limited to one rural and one urban site, these results support the conclusion that preemptive cutting of hemlock stands in rural areas may lead to greater losses of nitrogen compared to the slower decline caused by the hemlock woolly adelgid (Kizlinski and others 2002; Foster and Orwig 2006). This is because the stimulation of nitrogen losses caused by preemptive cutting is not reduced by plant uptake of nitrogen in rural sites where regrowth of vegetation is not as rapid as urban sites. However, this conclusion is limited by our experimental design which examined only one urban and one rural site. Also, there could be potential interactions between the hemlock woolly adelgid with the study treatments that makes direct comparisons between the rural and urban sites limited.

Nitrate Sources

Our values for $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in atmospheric inputs fall within the range of published literature values for isotopic values of precipitation

within North America and Europe (see Kendall and others 1996; Pardo and others 2004). We evaluated isotopic values of inputs during the snow-free season at both sites. Many studies in the United States suggest that $\delta^{18}O$ values of inputs are similar between NO_3^- in snow and rain (Kendall and others 1996; Burns and Kendall 2002; Campbell and others 2002). In those studies where $\delta^{18}O$ values are seasonally different, they tend to be higher in snow compared to rain (Pardo and others 2004). For our mixing model (equation (3) above), we used the mean value for $\delta^{18}O$ in atmospheric inputs (68 and 71‰ for the Arnold Arboretum and Harvard Forest, respectively) and in leachate (23 and 68‰ for the Arnold Arboretum and Harvard Forest, respectively) and the published range of $\delta^{18}O$ values for NO_3^- produced from nitrification (-5 to $+15$‰; see Kendall and others 1996; Pardo and others 2004). We calculated that between 62–85% and 4–6% NO_3^- in leachate was microbially produced at the Arnold Arboretum and Harvard Forest, respectively. We expected that a larger proportion of NO_3^- in leachate would be directly from atmospheric inputs at the Arnold Arboretum compared to Harvard Forest because it is located in an urban area and has relatively larger nitrogen inputs (Figure 1). If the microbial and plant demand for nitrogen was saturated by the large rate of nitrogen inputs at the Arnold Arboretum, one would expect a greater proportion of leached NO_3^- to come directly from atmospheric sources. Contrary to this expectation, a much smaller proportion of NO_3^- in soil leachate came directly from atmospheric inputs, rather than microbial processes, at the Arnold Arboretum compared to the Harvard Forest.

Rates of nitrogen inputs (Figure 1) and in leachate (Figure 2) are significantly lower at the Harvard Forest compared to the Arnold Arboretum. Under ambient levels of nitrogen deposition, previous studies have shown that both mixed hardwood and pine-dominated forest stands at Harvard Forest retain close to 100% deposited nitrogen (Magill and others 2004; McDowell and others 2004; Nadelhoffer and others 2004). At the time of this study, therefore, there is little evidence to suggest that biotic demand for nitrogen at Harvard Forest was saturated. With greater nitrogen retention at Harvard Forest, we would have expected any nitrogen that was lost in leachate to come primarily from nitrification, because we did not expect the biotic demand for these forests to be saturated. Other than the effect of cutting or girdling, we observed no obvious indication that forests were in decline at Harvard Forest to explain a

low biotic demand for nitrogen. Nitrate concentrations in leachate increased in the cut plots at Harvard Forest, but not until 2008 (Orwig and Lux, *personal communication*), which was 2 years after this study and 3 years after the experimental treatments began, suggesting that treatment did not directly affect nitrogen uptake at the time of this study. Relatively high $\delta^{18}O$ values of leachate NO_3^- are typically considered to be a symptom of nitrogen saturation because they reflect direct losses of NO_3^- that are not biologically processed. However, the results of this study suggest that systems with relatively high nitrogen retention can lose some NO_3^- that reflects an atmospheric source, suggesting that caution should be used if evaluating $\delta^{18}O$ values of NO_3^- alone without other measures of nitrogen retention or loss from an ecosystem.

There was no impact of treatment on $\delta^{18}O$ values of NO_3^- in atmospheric inputs or soil leachate at either of the two sites (Figure 4). There were treatment effects on $\delta^{15}N$ values in both NO_3^- inputs and soil leachate but the effect varied by site. The lack of a treatment effect on $\delta^{18}O$ values of NO_3^- in leachate suggests that treatment did not impact the source of NO_3^- lost from any of the forest stands we measured within the Harvard Forest or the Arnold Arboretum. The significantly higher $\delta^{15}N$ values in soil leachate within the hardwood control plots compared to the cut hemlock plots at Harvard Forest suggests that rates of nitrogen cycling may have been significantly higher in the hardwood plots, which is consistent with previous studies which have found greater rates of nitrogen cycling in hardwood compared to hemlock stands (Finzi and others 1998; Jenkins and others 1999; Templer and others 2003; Lovett and others 2004). Previous studies have shown a positive correlation between rates of soil nitrogen cycling and $\delta^{15}N$ values of soil as well (Templer and others 2007). The lack of a treatment effect on $\delta^{15}N$ values in soil leachate at the Arnold Arboretum suggests that there was not a strong difference in nitrogen cycling rates among plots there. However, the relatively higher $\delta^{15}N$ values of NO_3^- inputs within the control plots compared to the cut and chipped plots at the Arnold Arboretum could indicate greater rates of nitrogen transformations within the canopy of the control plots compared to the other plots. Alternatively, higher $\delta^{15}N$ values of NO_3^- inputs within the control plots could be a result of the contributions of insects, because animals generally have higher $\delta^{15}N$ values relative to other forms of nitrogen (DeNiro and Epstein 1981).

CONCLUSIONS

This study documents the interactive effects of an introduced pest, the hemlock woolly adelgid, management aimed at reducing the impacts of this pest and changes in the nitrogen cycle caused by differences in atmospheric inputs of nitrogen. Nitrogen inputs and losses were significantly greater at the urban site, the Arnold Arboretum, compared to the rural site, Harvard Forest. At the Arnold Arboretum, rates of nitrogen inputs to the forest floor were significantly greater in the control compared to the cuts plots. Surprisingly, our results show that NO_3^- in leachate at the Arnold Arboretum comes primarily from nitrification, whereas NO_3^- at the Harvard forest comes primarily from atmospheric deposition inputs. This suggests that ecosystems that lose a relatively small amount of NO_3^- can still have a significant proportion of that leached NO_3^- come directly from atmospheric deposition. Out study was limited to one rural and one urban site, but our results also suggest that current management regimes used to control the hemlock woolly adelgid, such as preemptive or salvage cutting, may lead to reduced nitrogen losses in urban areas if rapid regrowth of vegetation occurs to take up nitrogen. In contrast, preemptive cutting of trees in rural areas may lead to proportionately greater losses of nitrogen if vegetation regrowth is slow to occur, although the total magnitude is still smaller than in urban sites.

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