

# The impact of hemlock wooly adelgid infestation on understory community composition in an old-growth forest in western Virginia

*By*

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## Abstract

This study examines the legacy left by Eastern Hemlock (*Tsuga canadensis*) in understory plant communities following infestation by the Hemlock Woolly Adelgid (*Adelges tsugae*). Hemlocks strongly modify the understory environment and their removal may cause loss of native hemlock-associates and increased establishment of exotics. Forty plots were sampled, divided evenly across four types or phases of forest composition: A) healthy hemlock, B) yellow birch, C) adelgid-killed hemlock, and D) gap. The plots were distributed along three stream branches in an old-growth forest co-dominated by Eastern Hemlock and Yellow Birch (*Betula allegheniensis*) in the vicinity of Mountain Lake Biological Station, Virginia. Data describing floristic composition (species presence and frequency) and habitat location and conditions (e.g., distance from stream and soil pH) were collected for each plot. These data were analyzed to determine whether 1) more exotics occur in gaps and under dead hemlocks than in other plot types, 2) community composition differs among the four forest types, and 3) there are environmental differences among these plot types. It was found that there are more exotic species upstream (i.e., nearer but downstream from roads) and in gaps, the species-area curves of the four plot types differ, community composition under hemlocks, gaps, and birches map to ordination space in ways distinct from one another, soil beneath hemlocks and birches is distinctly different in such characteristics as pH, organic matter content, and plant nutrients, and the minimum daily temperature beneath live hemlocks is significantly warmer. These differences suggest there will be implications for the whole community as many large hemlocks are lost.

## Introduction

In the cove forests in western Virginia, many hemlock trees (*Tsuga canadensis*) are dying as a result of the Hemlock Woolly Adelgid (*Adelges tsugae*), an introduced insect that has already decimated many more northerly populations (Orwig and Foster 1998). The adelgid first arrived in the U.S. on the West Coast where it is a pest on Western Hemlocks, though it does not kill them. In 1951 the adelgid was accidentally introduced into Maymont Nursery in Richmond, VA. From there, it spread for nearly 50 years at a rate estimated at about 10 miles per year (Rhea 1994). More recently the adelgid appears to have greatly accelerated its rate of expansion to infest nearly all Appalachian hemlock stands south of Virginia over the course of a decade.

Adelgids feed on the sap of their host, which can result in total defoliation and death of the tree within two to five years of the parasite's arrival (Cheah et al. 2004). Because mortality is species-specific and many trees die suddenly at about the same time, invasion of the adelgid has effects outside of the ecosystem's natural range of variation in mortality (Busing 2005).

Because of earlier waves of introduced pests and diseases such as the American Chestnut Blight and the Balsam Woolly Adelgid, many of the largest trees still found in the southern Appalachian Mountains are in old-growth stands of Eastern Hemlock. Trees in these forests can be 200 to 400 years old, though only a few of these virgin pockets remain. The old-growth stands are often part of a unique ecosystem found in cove forests co-dominated by Eastern Hemlock and the hardwood Yellow Birch (*Betula allegheniensis*). The broader goal of this study is to understand how the character of these hemlock-birch co-dominated forests is changing as the hemlocks die.

There is no question that the forest community in adelgid-infested forests will change in significant ways due to such a massive, selective, and sudden loss of canopy. The change may be even more dramatic if corridors are opened for exotic species that are already present upstream of the forest. Furthermore, hemlock trees strongly modify the microenvironment in stands where they dominate. These modifications (and their reversal following hemlock death) may play a large role in determining the vegetation composition of the herb stratum beneath hemlocks. Eastern Hemlock fits the definition of an ecosystem engineer through its effects on light, temperature, soil chemistry, and soil structure. Hemlocks have dense, evergreen canopies that cause low light levels year-round on the forest floor. Both their needles and their wood are acidic and slow to decompose (Godman and Lancaster 1990). This slows cycling of plant nutrients like nitrogen, alters soil chemistry, and creates a tough organic mat or more humus layer on the forest floor (Finzi et al. 1998). Hemlock increases or maintains its dominance over time because it can tolerate the conditions it creates. These conditions affect a diverse range of other taxa as well, from warblers (Tingley et al. 2002) to ants (Ellison et al. 2005). Many of the ecologically important physiological and chemical characteristics of Eastern Hemlock are not shared by Yellow Birch, the canopy species most likely to replace it in forests where they are co-dominant. Yellow birch is relatively slow-growing and long-lived, but not to the extent that hemlock can be. It is deciduous, has less acidic leaf litter than hemlock, and is a gap-phase generalist (Erdmann 1990). It is unclear what the implications would be for the community if birch trees replace the majority of hemlock trees when they die.

Whether or not hemlocks will be replaced by birches decades from now, a more short-term and unavoidable result of adelgid infestation is a sudden increase in intense light reaching the forest floor due to defoliation of the canopy trees. This by itself can cause photoinhibition of shade-adapted plants (Houter and Pons, 2005). More light could also cause loss of soil moisture through increased evaporation, and higher daytime temperatures in the microclimate near the forest floor (Clinton, 2003). Defoliation leaves the area beneath the former canopy more exposed to the elements, and harder direct rain could cause erosion of organic material and leaching of nutrients. The newly exposed area is more insulated and less insulated, so temperature may vary over a greater range and there is less protection from wind and frost. These changes will most likely be a detriment to some species, and favorable for others (Rankin & Tramer 2002).

One major concern is that hemlock gaps will create an interim avenue of suitable establishment sites for exotic invasive species (Small, Small, & Dreyer 2005). Native sun-tolerant species that take advantage of forest gaps that occur in the normal dynamics of old-growth forests may be unable to compete with more aggressive exotics if they are present. Hemlock cove forests are associated with streams, and these may flow through disturbed areas such as roads or human settlements where there are many exotic species brought in with topsoil, mud on logging and other vehicles, and other human activities. Seeds from invasive species established in these disturbed sites may be carried downstream or down roads and paths by vehicles, people, and animals (Brown and Peet 2003). Light gaps left by the adelgid could provide more suitable habitat for weedy exotics whose seeds have been dispersed into the forest where before, the dense shade from live hemlocks acted to limit establishment (Loehle 2003). Once established within the forest, successful exotics may become new seed sources that promote further invasion.

A likely sequence of change in canopy structure in response to adelgid infestation is: 1) live hemlocks are defoliated and become newly dead hemlocks, 2) dead hemlocks fall or decompose and become gaps, 3) gaps are filled by birch since adelgids may continue to prey on new hemlock growth while birch seedlings preferentially establish on dead hemlock substrate (Marx 2005), and 4) the birch eventually dies and becomes a gap again. If removal of hemlocks from the system is already causing a release for some exotic species through fast-changing environmental variables like light penetration, there should be more exotic species found beneath dead hemlocks than live hemlocks. If removal of hemlocks from the system has the potential to cause release of exotics through slower-changing environmental variables like soil structure and soil chemistry, there should be more exotic species found beneath older gaps and birches. If there is a difference in vegetation community composition under healthy hemlocks, dead hemlocks, gaps, and yellow birches, adelgid infestation will impact a whole suite of associated species and change both the herbaceous and canopy strata. Differences in environmental variables under trees (or gaps) of the above categories could reveal the mechanisms responsible for changing vegetation composition, and could provide information about which environmental factors are the best predictors of the response of herbaceous vegetation to the removal of hemlocks. This study is designed to examine which of these scenarios are taking place in a forest where some hemlocks have been killed by the adelgid and some hemlocks still remain healthy for now.

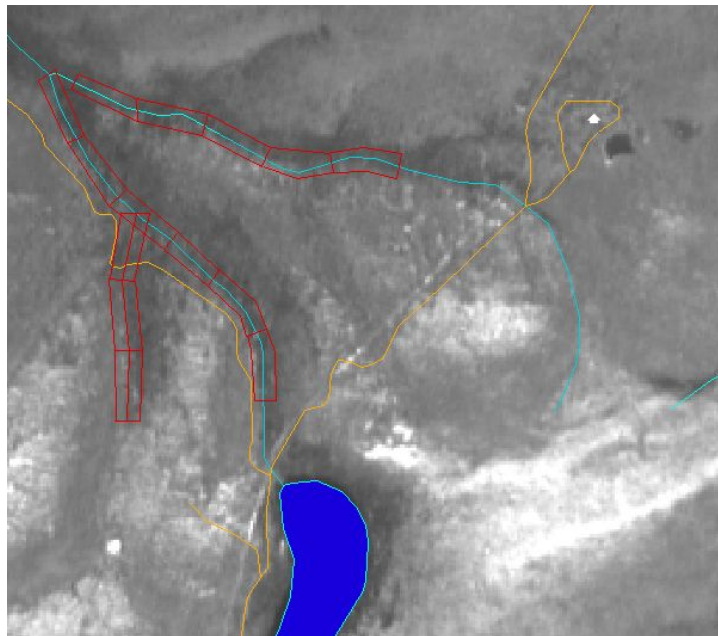
## Methods

### *Study site*

The study site consisted of three watersheds (Pond Drain, Hunter's Branch, and Hogskin Branch) near Mountain Lake Biological Station (University of Virginia), on Salt Pond Mountain in Giles County, VA. All three have roads at the top; both Pond Drain and Hunter's Branch intersect Giles county road 613 near their headwaters, and the top of Hogskin Branch intersects an unpaved road. Pond Drain runs parallel to an infrequently used unpaved road for most of its course down the mountain. Hunter's Branch has a road and disturbed area for the first 0.5 km downstream from road 613. Each branch is similar in upstream source of invasive plants. The three branches differ somewhat in slope, aspect, terrain, and understory community, but they all run through old growth forests where numerous large hemlocks have been killed by adelgids.

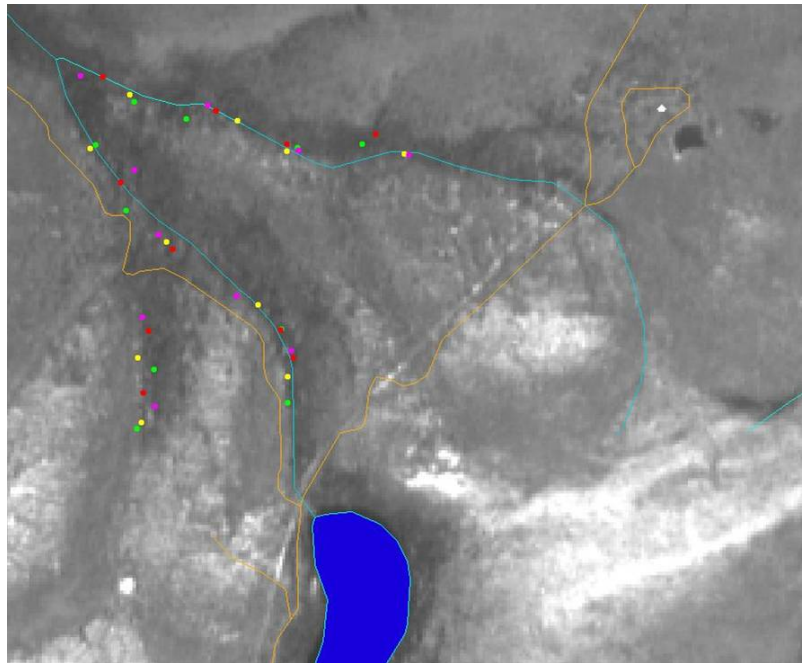
### *Sampling Design*

The three branches were divided into a series of adjacent zones that each extend 250 m along the stream. The zones begin at the edge of old-growth forest and continue downstream until the branches intersect each other. Pond Drain was divided into six zones, Hunter's Branch was divided into five, and Hogskin Branch was divided into three (Figure 1). All zones are numbered beginning at each branches' upstream edge. Due to time constraints, the third zone in each branch and the sixth zone of Pond Drain were not sampled, leaving a total of ten zones.



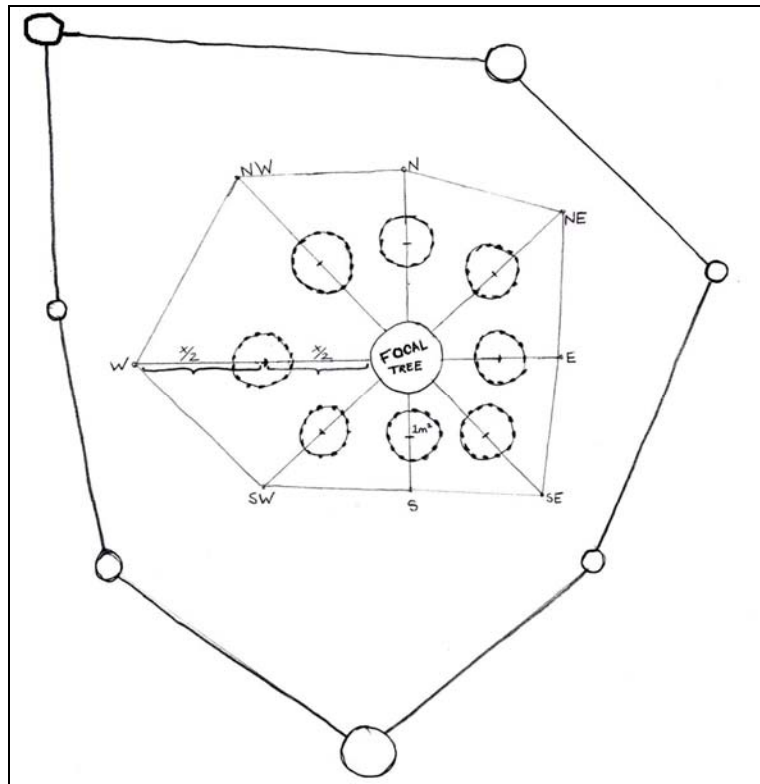
**Figure 1.** Location of zones along the down-stream gradient for three streams in the study site. From top to bottom the streams are Hunter's Branch, Pond Drain, and Hogskin Branch.

Within each zone, we randomly selected a tree or gap from suitable options in each of four categories: A) large healthy hemlocks, B) large healthy birches, C) hemlocks that have recently died from the effects of adelgids, and D) gaps. There were several criteria for suitable focal trees. Hemlocks were either quite healthy or completely dead- no intermediates were used. The tree's diameter at breast height was greater than 55 cm. Gaps resulted from the loss of one main canopy tree, though the gap-forming trees were of different species and in various stages of decay. Focal trees had to fall clearly within one of the four categories- live trees were selected when the canopy around them was closed when possible so that their understory community wasn't influenced by a nearby gap, and double-trunks of adjacent, nearly touching birches and hemlocks were not used. Trees were selected at varying distances from the stream but closer than 40 m whenever possible. The selected tree or gap designated the location of a plot, the fundamental data collection unit of this study. Four plots in each of ten zones made a total of forty plots spread across the three branches, distributed along the downstream gradient (Figure 2).



**Figure 2.** Location of each of the forty plots. Live hemlocks are green, dead hemlocks are red, birches are purple, and gaps are yellow.

The size and shape of a plot was determined by the canopy of the focal tree and by the arrangement of its nearest neighbors. A polygon was formed by creating eight axes in the cardinal compass directions, each extending from the base of the focal tree to the edge of its canopy defined using a right-angle prism with an integrated level, or to the edges of the neighboring trees' canopies in the case of gaps (Brokaw 1982). In gap plots, when it was unclear where the base of the main tree would have been or when some sides of gaps were considerably grown over, a flag was placed in the center of the gap to serve as the center point for the radii of the polygon. The polygon that delimited the edge of the canopy was nested inside a larger polygon formed with the bases of neighboring canopy trees as vertices (Figure 3) (Runkle 1981). This is an attempt to account for the fact that not all light entering the plot is perpendicular so the canopy may affect an area larger than its vertical projection on to the ground, at least with respect to shade.



**Figure 3.** Diagram of a typical plot layout. Solid circles are neighboring canopy-tree bases. Dashed lines are the locations of 1 m<sup>2</sup> plots at ½ the distance to the canopy edge of the focal tree along each cardinal compass axis.

At each plot, we collected the following data:

- **Plot slope** from the highest to lowest point in the plot using an Abney level, and **plot aspect** using a sighting compass. These provided information about potential differences in light angle, runoff, and weather effects at the different sites
- **Plot location** with a handheld Magellan Model 315 GPS unit to determine where the focal tree is located with respect to the roads, other plots, and its distance downstream
- **Distance and direction to stream and height above stream bed** (using the slope from the focal tree to the stream) with SONIN® range finders, a sighting compass, and an Abney level to estimate the stream's potential as a source of seeds and disturbance, as well as the soil moisture conditions at the plot
- **A soil sample** pooled from the first six inches of soil at the 8 vertices of the inner polygon. In addition to the soil samples taken on each plot, a second set of sixteen soil samples were taken using a factorial design in which soil was collected from beneath pure stands of Hemlocks and pure stands of Birch in both upper Pond Drain and lower Pond Drain. There were four replicates in each species-by-site quadrant. These samples were taken in order to determine more straight-forwardly if hemlocks

and birches create different soil conditions because plot soil samples were not taken in pure stands. All samples were sent to the Soil Analysis Laboratory at Virginia Polytechnic Institute and State University where *pH*, organic material, cation exchange capacity, and several macro and micronutrients necessary for plants were measured to provide information about the soil's role in determining plant community composition

- **Direction and distance to edge of focal tree canopy or gap** using a sighting compass and SONIN® range finders to describe shape and extent of the gap or canopy
- **DBH of focal tree** using a DBH tape-measure, and in the case of gaps, the probable cause of the gap and its relative age estimated by the type and size of sprouts growing from the stump and the extent to which the stump had rotted.
- **Presence of all vascular plant species in the inner and outer polygons** that could be detected with a thorough search and **cover values for all species present in eight 1m<sup>2</sup> samples within the plot** by placing a 1m<sup>2</sup> hoop halfway to the canopy edge along each of eight cardinal directions and estimating percent cover of every species within the hoop to describe community composition in different plots. Unknown species were collected and identified using keys in Wofford (1989), Radford et al. (1968) Strausbaugh and Core (1977), and Weakley (2005). Botanical nomenclature follows Weakley 2005. Alan Weakley, Curator of the University of North Carolina Herbarium (NCU), confirmed many identifications. Voucher specimens are deposited in the herbaria of Mountain Lake Biological Station or the University of North Carolina.

### *Temperature Loggers*

Sixteen iButton® temperature sensors were placed throughout the study area, four in each type of plot. These were located in zone 1 of Hunter's Branch, zone 1 and 4 of Pond Drain, and zone 2 of Hogskin Branch. They were calibrated using a hot-water bath and programmed to record ambient temperature every half-hour for one month from June 29th to July 29th. They were inserted into a PVC pipe 2.5 cm in diameter and 10 cm long that was placed halfway to the north edge of the canopy of the chosen tree.

### *Mustard Survey*

To specifically address the question of whether there is potential for exotic species to move downstream into the site from roadside sources, a supplementary study was conducted on the distribution of two exotic mustards: *Barbarea vulgaris* and *Alliaria officinalis*. This was done in early June (while both species had conspicuous flowers) by walking down the three branches of the study site beginning at their intersection with the road and counting all mustard plants visible from the stream. Sonin® range-finders were used to produce totals for both species every ten meters. A handheld GPS unit was used to provide location coordinates every 100 m and to plot the location of each plant once they became less frequent.

## Results

Results will be presented in three categories to address my three questions: 1) Are there more invasive exotics in gaps and under dead hemlocks than in other types of plots? 2) Is there a difference in community composition under healthy hemlocks, dead hemlocks, yellow birches, and gaps? 3) Are there differences in environmental variables under trees of the above categories?

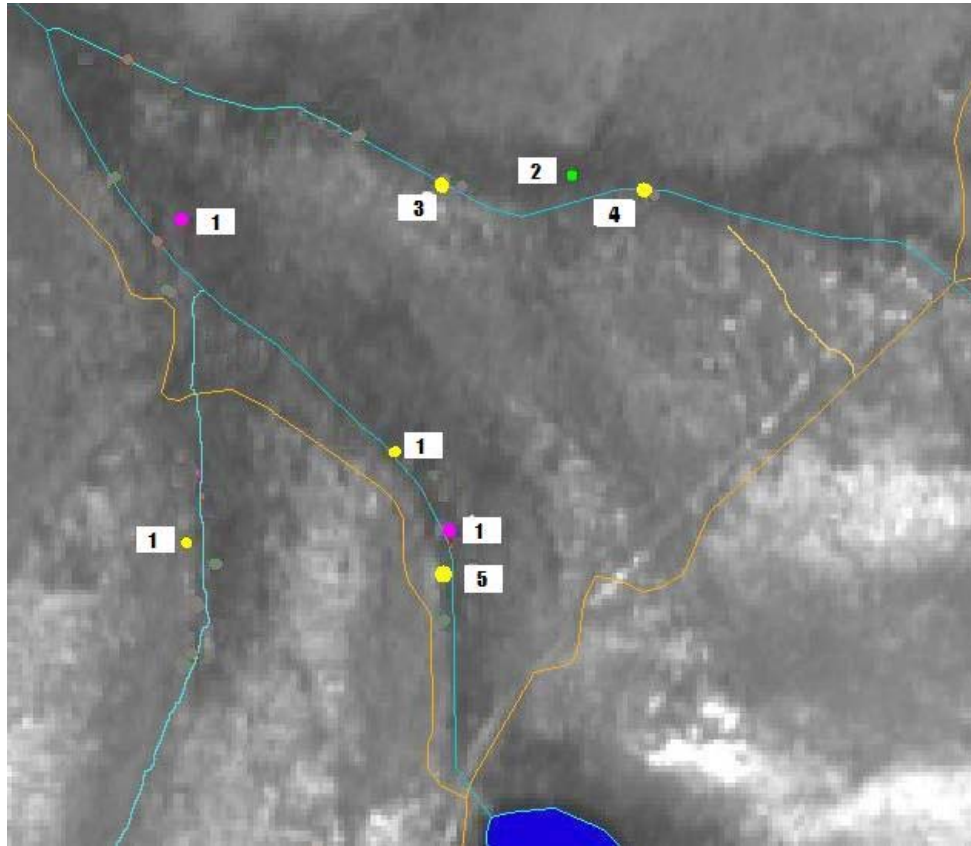
### Exotic Species

#### *Plots*

More than 125 species were identified in the forty plots studied. Of these, seven were weedy exotics; *Rumex crispus*, *Prunella vulgaris*, *Cerastium glomeratum*, *Barbarea vulgaris*, *Veronica sp.*, *Rosa multiflora*, and *Persicaria cespitosum*. Excluding *Persicaria*, eight out of forty plots contained at least one exotic species. The pattern of occurrence of these species is summarized in Table 1 and Figure 4. *Persicaria cespitosum* was definitely in the plots and may have been the most abundant invasive, but unfortunately I did not discriminate when taking data between this exotic species and a native relative (*Persicaria punctatum*) which also occurred in the plots. The most abundant exotic consistently identified to species was *Prunella vulgaris*. However, there is some question about whether *Prunella vulgaris* is introduced or native (Nelson 1962). Exotic species were found more frequently in the upstream portions of the study area. Five of the eight plots containing exotic species were gaps, two were birch plots, and the last was a live hemlock plot in which one corner was a small streamside gap\* and this was where the exotic species were found.

**Table 1.** Location, Number, and Types of Exotic Species Found in Plots. PD = Pond Drain, HS= Hogskin Branch, and HB= Hunters Branch. Zone numbers increase with distance downstream from 1 to 5.

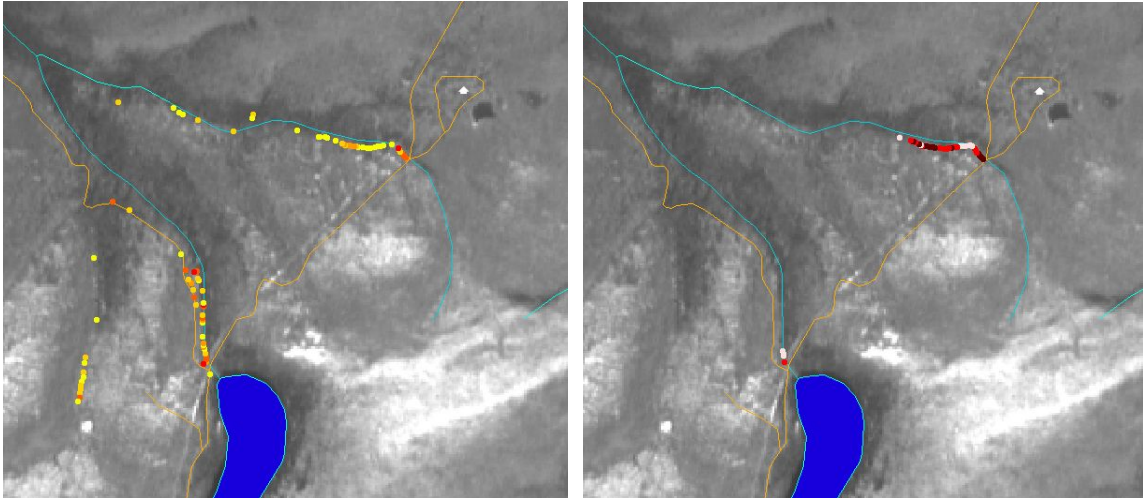
Location (Zone-Branch)	Number of Exotic Species	Exotic Species	Plot Category
5-PD	1	<i>Prunella vulgaris</i>	Birch
2-PD	1	<i>Prunella vulgaris</i>	Gap
2-HS	1	<i>Barbarea vulgaris</i>	Gap
1-PD	1	<i>Rosa multiflora</i>	Birch
1-HB	2	<i>Prunella vulgaris</i> , <i>Rumex crispus</i>	Live Hemlock*
2-HB	3	<i>Prunella vulgaris</i> , <i>Cerastium glomeratum</i> , <i>Barbarea vulgaris</i>	Gap
1-HB	4	<i>Prunella vulgaris</i> , <i>Cerastium glomeratum</i> , <i>Barbarea vulgaris</i> , <i>Rumex crispus</i>	Gap
1-PD	5	<i>Prunella vulgaris</i> , <i>Cerastium glomeratum</i> , <i>Barbarea vulgaris</i> , <i>Veronica sp.</i> , <i>Rumex crispus</i>	Gap



**Figure 4.** Locations of all the plots where exotic species were found and the number of species found in each plot. Yellow dots are gaps, purple dots are birches, and green dots are hemlocks.

#### *Mustard Surveys*

The survey of introduced mustard occurrence along the three branches of the study area showed the majority of *Barbarea vulgaris* was concentrated in the upper portions of the branches, though it was found occasionally in open areas downstream as well (Figure 5). *Alliaria officinalis* was restricted to the sections of Pond Drain and Hunter's Branch closest to Road 613, and in the places it had become established it was more dense and abundant than *B. vulgaris*. *A. officinalis* was also established in places where the canopy was completely closed, unlike *B. vulgaris* which appeared more in open places with some disturbance from the stream. Overall, fewer exotic species were found in the old-growth area where the forty plots were located than in the secondary forest upstream.

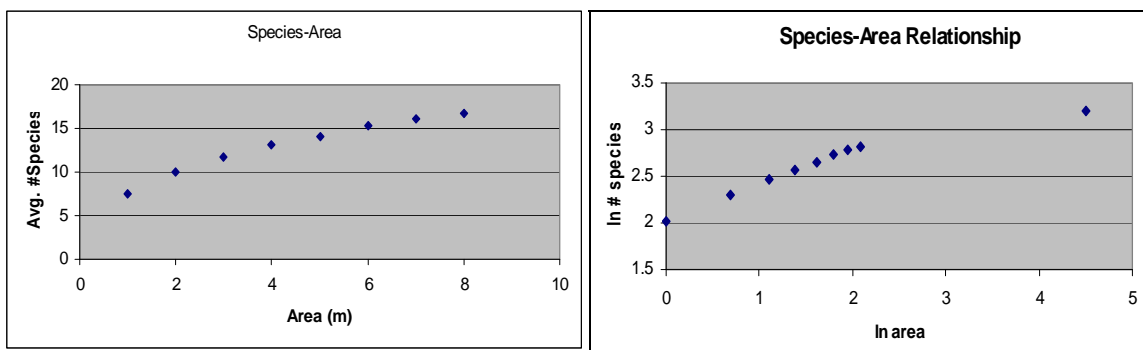


**Figure 5.** The downstream distribution of Yellow Rocket (*Barbarea vulgaris*) on the left and Garlic Mustard (*Alliaria officinalis*) on the right. Darker colors indicate more stems at that location. Yellow Rocket presence was also mapped along the road running parallel to Pond Drain. The last dark point on the road shows the site where construction vehicles made a clearing and dug a well.

## Community Composition

### *Effectiveness of sampling technique*

To test how effective the eight  $1\text{m}^2$  samples were at characterizing the vegetation of an entire plot, species-area curves were constructed for each plot by counting the number of species present in the eight samples of one hoop, the eight possible samples of adjacent two-hoop combinations, the eight possible samples of adjacent three-hoop combinations, and so on until the single possible eight hoop combination. These were averaged over the forty plots. Only 0.7 species on average were added with the eighth square meter. The mean area of the inner polygon for all forty plots was  $90.3\text{m}^2$ . In moving from a sample size of  $8\text{m}^2$  to  $90.3\text{m}^2$ , the average species richness increases from 16.8 species to 24.6 species (Figure 6).

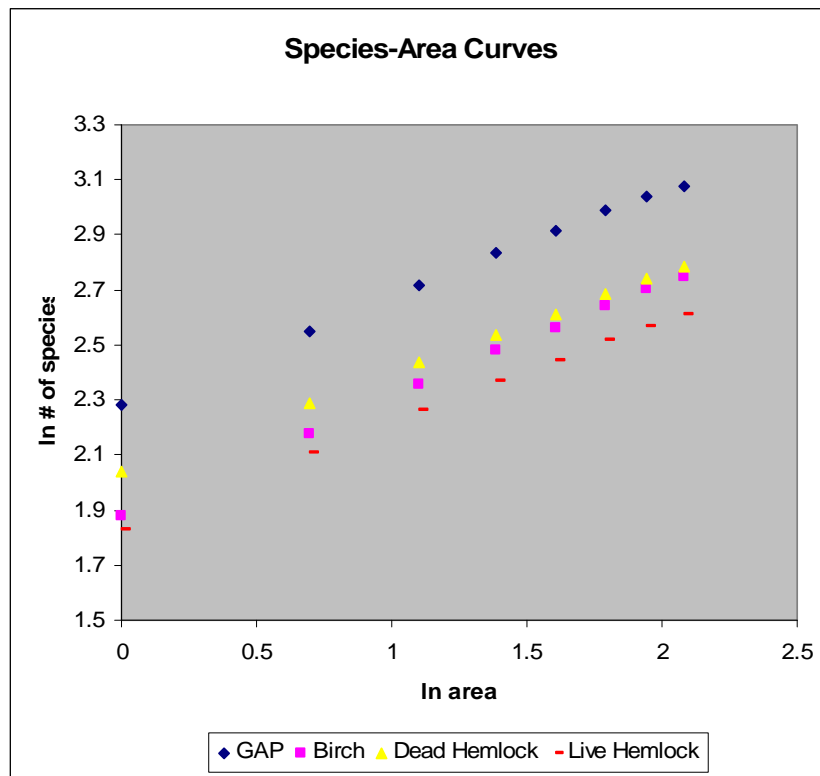


**Figure 6.** On the left, average number of species found in one, two, three, four, five, six, seven, and eight  $1\text{m}^2$  hoop samples of vegetation. On the right, a log-log plot of the species richness from 1 to  $8\text{m}^2$  along with the average species richness for the entire inner polygon. The

regression line from eight samples would significantly overestimate the species richness in the entire polygon, so most of the species in the polygon are sampled in the eight 1 m<sup>2</sup> hoop samples.

### Species-Area Curves

The species-area curves produced by averaging the ten plots within each category showed the four types of focal trees had significantly different patterns of species richness (ANCOVA test with equal slopes but different intercepts;  $F_{3,24} = 938.5$   $p < 0.0001$ ), with live hemlocks having the lowest number of species, followed by live birches, dead hemlocks and, and gaps respectively (Table 2) (Figure 7). The dead hemlocks gained species at a slower rate with increasing area than the other three categories and birches showed the greatest change in species with area.



**Figure 7.** Log-log species-area curves for each of our four focal tree types.

**Table 2.** Regression Equation Values Accompanying the Species-Area Curves in Figure 7.

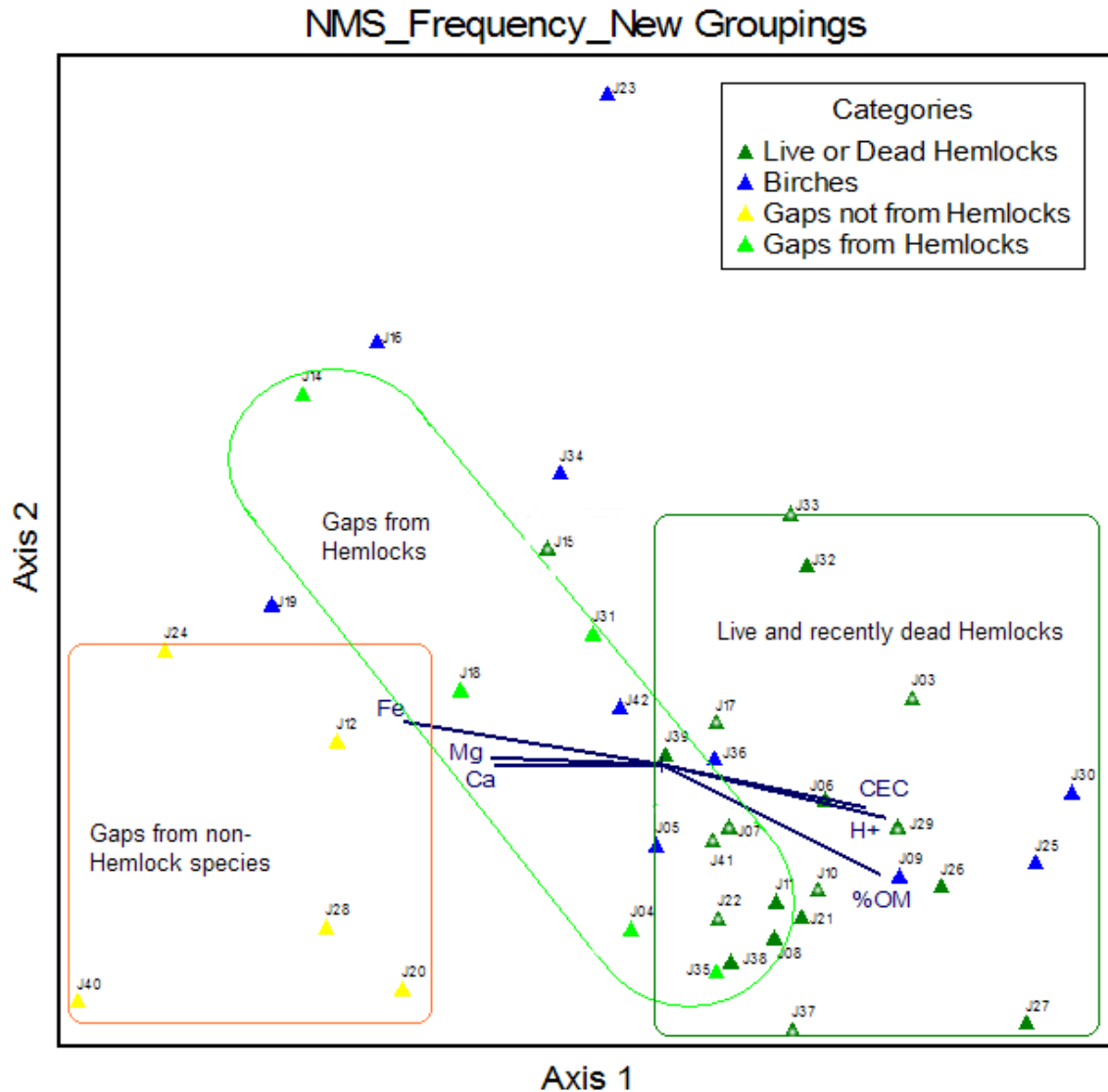
	y-intercept	slope	r <sup>2</sup>
Live hemlocks	1.83979	0.37589	0.999
Dead hemlocks	2.03912	0.35984	0.999
Birches	1.88568	0.42072	0.999
Gaps	2.28553	0.38859	0.999

### *Ordination and Cluster Analysis*

Nonmetric Multidimensional Scaling analysis was performed on the vegetation data using 39 of the plots and the 76 species that occurred in the hoop samples. The fortieth plot was a live hemlock from upper Hunter's Branch that bordered a gap and had herb-layer vegetation dominated by a New York Fern patch, making its appropriate classification ambiguous, so it was not included in the analysis. For each species in each plot, the number of 1.0 m<sup>2</sup> circular subplots (0-8) where the species was present was used as the importance value. Data was analyzed using PC-ORD Ver. 5 software following the method from Mather (1976) and Kruskal (1964) employing the Sorensen distance measure. Results were generated using a random starting configuration and 50 runs with real data.

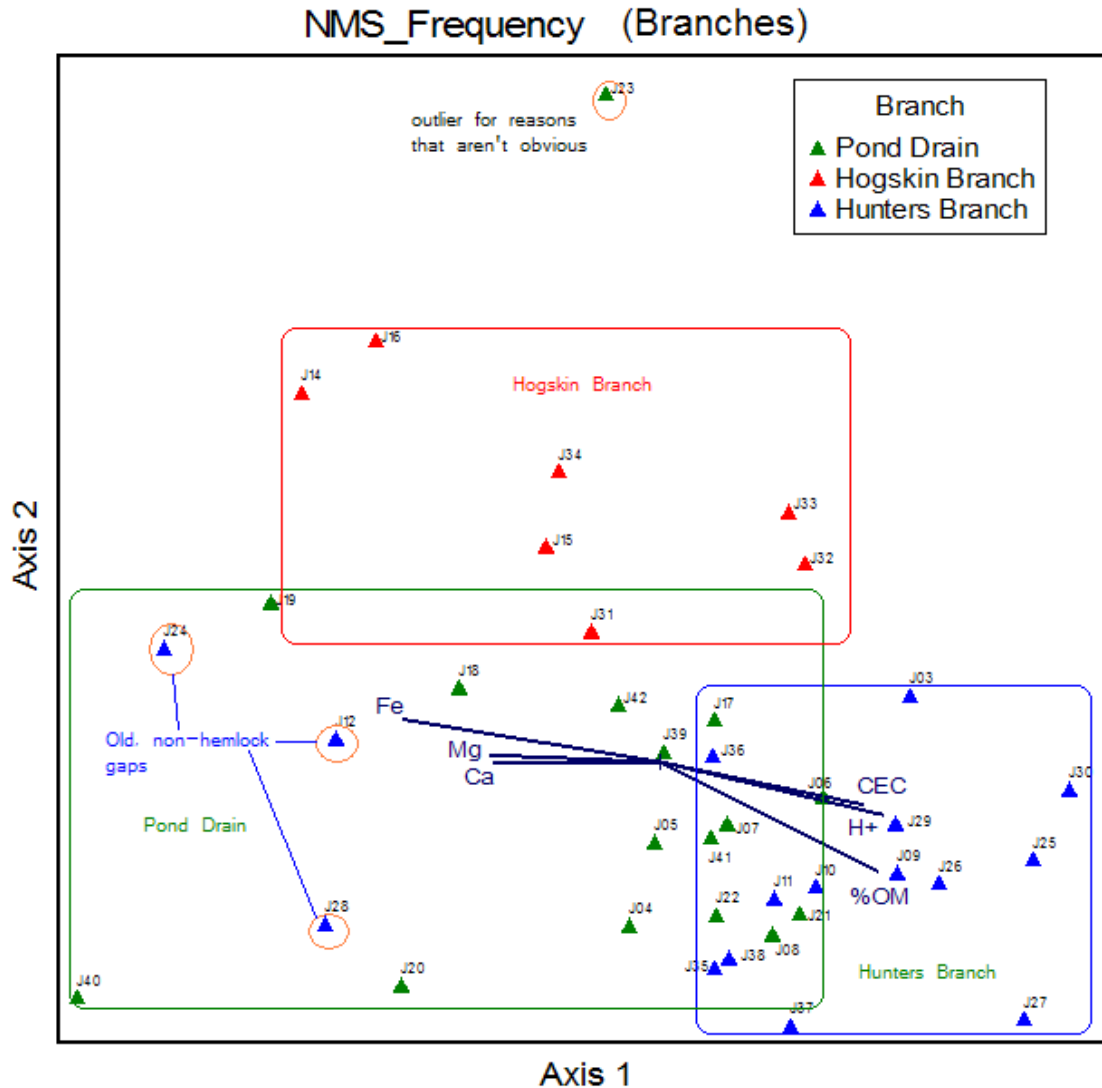
The final 2-dimensional solution was produced after 250 iterations. The final instability was 0.00056. The proportion of variance represented by each axis based on the r<sup>2</sup>-value between distance in the ordination space and distance in the original space was 0.628 and 0.214 for axis 1 and 2 respectively, for a total proportion of variance of 0.843 explained with the first two axes. The final stress was 17.04 and a Monte Carlo test showed the proportion of randomized runs with stress less than or equal to the observed stress was 0.0040, indicating that axes are significantly stronger than those expected by chance.

Though the plot categories were originally live hemlocks, dead hemlocks, birches, and gaps, ordination revealed that the live and recently dead hemlocks sampled had similar herb-stratum vegetation patterns, whereas the group "gaps" had distinct vegetation patterns internally depending on whether or not the gap was from a hemlock tree. For subsequent analysis of vegetation data, live and dead hemlocks were lumped into a single group and gaps were split into two groups: gaps made by hemlocks and gaps made by hardwoods. Figure 8 shows the arrangement of these groups in ordination space. Gaps made by hemlocks are generally between gap plots and hemlock plots. Birch plots are scattered throughout the ordination space. Figure 8 also shows the gradient for several environmental variables between hemlocks and gaps. Hemlock plots have high soil acidity, high percent organic matter, high cation exchange capacity, and low concentrations of the nutrients Iron, Magnesium, and Calcium, while gap plots are the opposite.



**Figure 8.** NMS ordination of species frequency in the eight hoops per plot, colored by category. A small white dot in a dark green triangle indicates a recently dead hemlock. Dark blue lines show vectors for gradients in environmental variables. Rectangles are drawn to roughly suggest groupings.

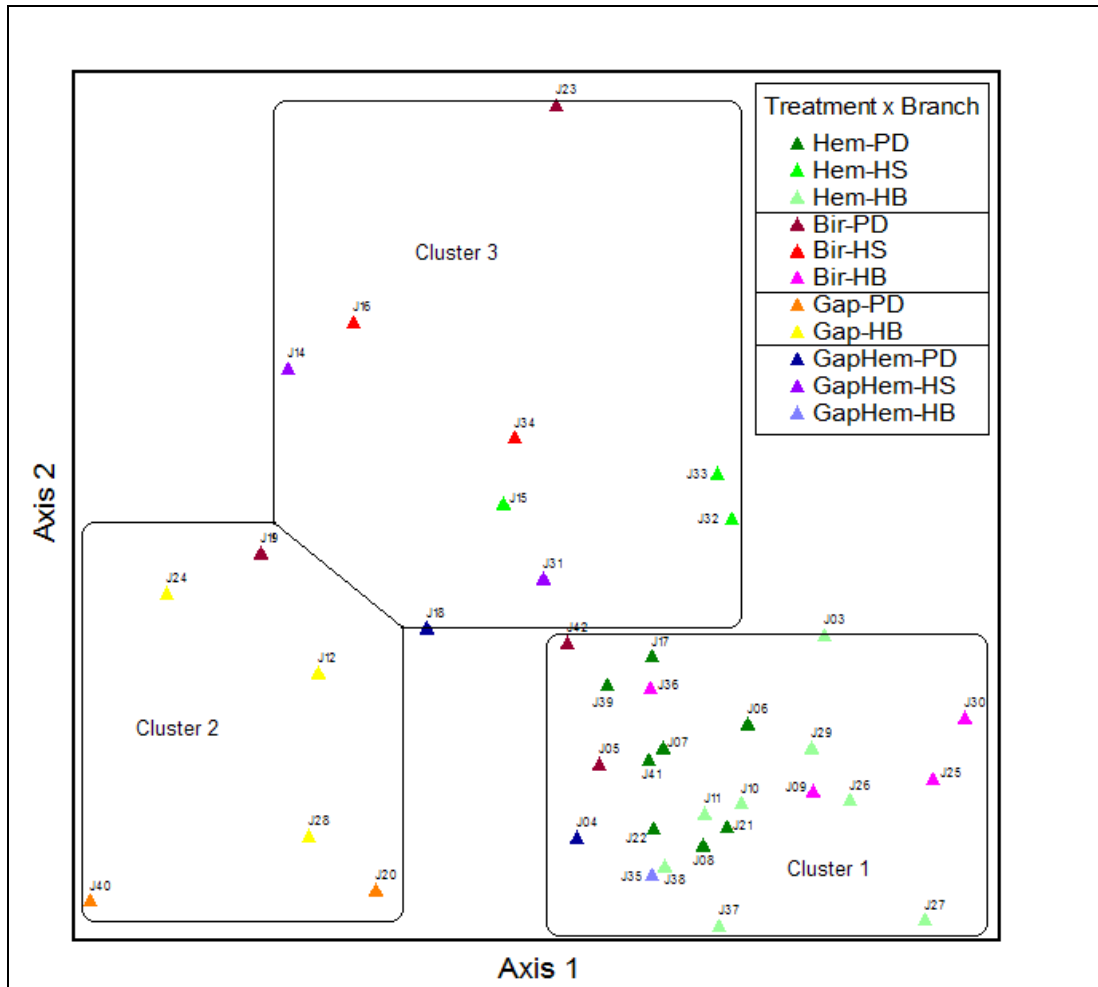
The same ordination of plots is shown in Figure 9 below, but the plots are labeled by the branch where they were located rather than the category of their focal tree. This shows that Hogskin Branch plots all cluster in a different area than the plots in Pond Drain and Hunters Branch, perpendicular to the environmental gradient vectors. Hunters Branch plots cluster in the high acidity, high organic matter, high cation exchange content end of the gradient for environmental variables, except for the non-hemlock gap plots. Pond Drain is somewhat intermediate between the two branches in ordination space, which is consistent with its geographic position.



**Figure 9.** NMS ordination of species frequency in the eight hoops per plot, colored by branch. Dark blue lines show vectors for gradients in environmental variables. Rectangles are drawn around areas where one branch dominates the ordination space, and outliers are noted with circles. Plot J23 contained an unusually high number of species and high habitat heterogeneity including a steep bank, a lower streamside portion, and an elevated terrace.

In order to compare my *a priori* categories with groups recognized by an unbiased method, Hierarchical Cluster Analysis was performed using the Sorensen distance measure and the Flexible Beta linkage method with a flexible beta value of -0.250. The resulting dendrogram was used to define three clusters within the plots. Pruning the dendrogram at three groups retained about 33% of information.

These clusters are shown in Figure 10 below. When both the identity of the focal tree and the branch location are taken into consideration, Group 3 from the cluster analysis corresponds very closely to plots from Hogskin Branch (no matter what category), Group 2 corresponds very closely to gaps made by hardwoods, and Group 1 corresponds to live hemlocks, dead hemlocks, gaps recently formed by hemlocks, and the birches in Hunters Branch.



**Figure 10.** NMS ordination of species frequency in the eight hoops per plot, colored to show both category and branch. Shades of green represent hemlocks, shades of red represent birches, shades of blue represent gaps from hemlocks, and shades of yellow represent gaps from hardwoods. Dark colors are Pond Drain, medium colors are Hogskin Branch, and light colors are Hunters Branch. Rectangles encompass plots in clusters generated by Hierarchical Cluster Analysis with three groups.

Indicator Species Analysis was performed on the clusters generated by the Hierarchical Cluster Analysis routine in PC-ORD. Indicator values were calculated with the method of Dufrene and Legendre (1997). These are reported in Table 3 with the Monte Carlo test of significance of observed indicator value for each species.

The results of the Indicator Species Analysis show that tree and shrub seedlings make up the indicator species for Cluster 1 (corresponding most with hemlocks). Cluster 2 (corresponding with gaps made by hardwoods) has the greatest number of indicator species with a significant observed indicator value. These are all herbaceous and include grasses and sedges. Additionally, three of the seven exotic species found in plots were indicators for this group. Cluster 3 (corresponding with plots in Hogskin Branch) consisted of herbaceous species as well— including many that form woodland ground cover such as *Anemone quinquefolia* and *Oclemena acuminata*.

**Table 3.** Results of Indicator Species Analysis using clusters generated by Hierarchical Cluster Analysis for species with significance values less than 0.1. Bold-face indicates values less than 0.05.

SPECIES	Cluster	Observed Indicator Value	p-value
<i>Prunus serotina</i>	1	<b>62.6</b>	<b>0.0002</b>
<i>Betula allegheniensis</i>	1	<b>46.1</b>	<b>0.0044</b>
<i>Amelanchier arborea</i>	1	<b>56.1</b>	<b>0.0082</b>
<i>Rhododendron maximum</i>	1	<b>46.7</b>	<b>0.0448</b>
<i>Ilex ambigua</i>	1	36.5	0.0532
<i>Magnolia accuminata</i>	1	37.9	0.0642
<i>Oxalis sp. (yellow flower)</i>	2	<b>66.7</b>	<b>0.0002</b>
<i>Scutellaria saxatilis</i>	2	<b>75.6</b>	<b>0.0002</b>
<i>Carex sp. (broad leaf)</i>	2	<b>66.7</b>	<b>0.0002</b>
<i>Glyceria sp.</i>	2	<b>96.8</b>	<b>0.0002</b>
<i>Boykinia aconitifolia</i>	2	<b>59.4</b>	<b>0.0004</b>
<i>Lycopus virginiana</i>	2	<b>66.7</b>	<b>0.0004</b>
<i>Polystichum achrostichoides</i>	2	51.3	0.0006
<i>Impatiens capensis</i>	2	61.4	0.0006
<i>Prunella vulgaris</i>	2	50	0.0014
<i>Cerastium glomeratum</i>	2	50	0.002
<i>Galium tinctorum</i>	2	46.5	0.0038
<i>Unknown Grass</i>	2	54.3	0.0066
<i>Thelypteris noveboracensis</i>	2	53.4	0.01
<i>Viola sp.</i>	2	51.3	0.0174
<i>Barbarea vulgaris</i>	2	33.3	0.0202
<i>Pilea pumila</i>	2	35.6	0.032
<i>Carex sp. (narrow leaf)</i>	2	35.2	0.0552
<i>Maianthemum canadense</i>	3	<b>75.9</b>	<b>0.0002</b>
<i>Anemone quinquefolia</i>	3	72	0.0004
<i>Mitchella repens</i>	3	61.1	0.0022
<i>Athyrium filix-femina</i>	3	42.2	0.0106
<i>Oclemena acuminata</i>	3	54	0.0116
<i>Trautvetteria carolinensis</i>	3	30	0.0392
<i>Circaea alpina</i>	3	36.3	0.0408
<i>Medeola virginiana</i>	3	29.7	0.0532
<i>Fagus grandiflora</i>	3	34.3	0.0762
<i>Veratrum viride</i>	3	20	0.0826

## Environmental Variables

### *Hemlock-Birch Soil Samples*

The soil beneath live hemlocks and live birches differed significantly in pH, organic matter content, cation exchange capacity, and parts per million of the plant nutrients Potassium, Copper, Iron, and Magnesium (Tables 4 and 5). The upstream (PDZ1) and downstream (PDZ4) portions of Pond Drain differed significantly in pH, Potassium, Calcium, Magnesium, and Zinc.

**Table 4.** Means and Standard Deviations of Soil Nutrients under Hemlocks (TSCA) and Yellow Birch (BEAL). Elements are reported as mg/kg, organic matter (OM) as percent dry weight on ignition, and cation exchange capacity (CEC) as milliequivalents per ml.

Site	Species	pH	SE	OM	SE	P	SE
PDZ1	BEAL	5.668	0.067	18.100	1.745	2.000	0.000
PDZ1	TSCA	4.830	0.076	71.200	5.409	2.000	0.000
PDZ4	BEAL	5.185	0.156	26.125	10.896	2.250	0.250
PDZ4	TSCA	4.783	0.050	57.325	10.583	3.000	0.707

Site	Species	K	SE	Ca	SE	Mg	SE
PDZ1	BEAL	28.800	1.828	460.800	43.118	58.200	7.046
PDZ1	TSCA	17.000	1.000	280.500	32.966	28.500	1.555
PDZ4	BEAL	20.000	2.415	211.000	36.667	21.000	2.799
PDZ4	TSCA	15.500	0.866	268.250	37.506	25.750	3.065

Site	Species	Zn	SE	Mn	SE	Fe	SE
PDZ1	BEAL	3.500	0.362	24.440	6.286	55.260	11.208
PDZ1	TSCA	3.125	0.342	35.800	13.920	6.500	1.255
PDZ4	BEAL	1.500	0.381	22.800	5.598	41.300	10.668
PDZ4	TSCA	1.725	0.293	21.675	4.865	12.725	4.280

Site	Species	Cu	SE	CEC	SE
PDZ1	BEAL	0.680	0.132	7.180	0.350
PDZ1	TSCA	0.225	0.048	10.950	0.411
PDZ4	BEAL	0.650	0.087	8.500	1.050
PDZ4	TSCA	0.375	0.103	11.175	0.287

**Table 5.** F tests of the effect of species (Eastern Hemlock or Yellow Birch), site (Upper or Lower Pond Drain), and their Interaction. All F statistics are on 1 and 13 Degrees of Freedom. F statistics that are significantly greater than 1 with a probability of Type I error < 0.05 are in boldface.

	Species	Site	Interaction
pH	<b>43.65</b>	<b>7.98</b>	<b>5.38</b>
Organic Matter	<b>30.58</b>	0.15	2.06
P	1.14	3.17	1.14
K	<b>23.04</b>	<b>9.20</b>	4.62
Ca	2.49	<b>11.29</b>	<b>9.28</b>
Mg	<b>6.93</b>	<b>17.78</b>	<b>13.22</b>
Zn	0.05	<b>23.28</b>	0.72
Mn	0.38	0.90	0.56
Fe	<b>19.88</b>	0.20	1.35
Cu	<b>12.30</b>	0.33	0.75
CEC	<b>30.42</b>	1.75	0.88

*Temperature Loggers*

The temperature sensors revealed that live hemlocks had slightly but significantly warmer minimum daily temperatures than birches (second warmest), dead hemlocks, and gaps using a one-way analysis of variance (Tables 6 and 8). Maximum daily temperatures showed that although live hemlocks had a much higher average temperature, there was too much variance within categories to detect significant differences between categories (Tables 7 and 8).

**Table 6.** ANOVA of Mean Daily Minimum Temperatures (°C)

	DF	SS	MS	F	P
Group means	3	5.125	1.7083	4.69	<b>0.0217</b>
Within groups	12	4.375	0.3646		
Total	15	9.500			

**Table 7.** ANOVA of Mean Daily Maximum Temperatures (°C)

	DF	SS	MS	F	P
Group means	3	100.5	33.500	1.96	0.1745
Within groups	12	205.5	17.125		
Total	15	306.0			

**Table 8.** Group Means

Groups	Minimum Temperature	Maximum Temperature
Live hemlock	10.125	28.50
Birch	9.375	22.00
Dead hemlock	8.750	23.25
Gap	8.750	23.35

## Discussion

### Exotic Species

Gaps were found more likely to contain exotic species than all other types of plots, and three exotic species were actually indicator species of gaps made by hardwoods. However, no exotic species were found in any plots where dead hemlocks were the focal tree. It is unclear whether the gaps left by adelgid-killed hemlocks will provide more suitable habitat for exotic species as they age, but it appears as though increased light due to canopy defoliation is not enough by itself to induce establishment by exotic species. Hemlocks may limit establishment in other ways, such as soil chemistry and structure of leaf litter, and these effects could persist longer than the few years that have passed since adelgids killed the hemlocks in the study site.

There are currently few invasive exotic species in the old-growth part of the forest where the plots were located. However, as shown in the mustard survey, there are exotic species poised just upstream and already there are signs of infiltration. Though a species distribution at one time is not enough evidence to say conclusively that a species is moving downstream from its source at the road, the patterns of occurrence suggest that both species are advancing. The drop in occurrence frequency upon entering old-growth forest may be because seeds have not yet dispersed so far downstream in great enough quantities for substantial establishment. On the other hand, different conditions in old-growth forests may be impeding establishment, even if plenty of seeds are available. Rates of establishment, survival, and growth of vegetation are correlated with characteristics of individual species such as dispersal ability and seed size (Chambers and MacMahon 1994), so we would expect different species of exotics to have different levels of success in establishment. It would be interesting to compare the results of this survey with surveys done in future years. Garlic Mustard has only been noticed on Salt Pond Mountain in the past few years, and in that time it has already aggressively colonized the upper portions of two of the branches in the study site. Because of its tolerance for shade, this species may be able to infiltrate hemlock stands regardless of whether they are infested by the adelgid, unless hemlocks create substrate that is unsuitable.

Overall, exotic species are found in gaps but have not yet invaded the gaps created by hemlocks, nor have they substantially invaded the old-growth portions of the branches as a whole. Further study is required to determine why this is and whether there is potential for invasion in the future. This will depend on the species available at the upstream source, their dispersal mechanisms, their requirements for establishment and growth, and the rate at which the ecosystem engineering effects of hemlocks are lost with the death of hemlocks.

### Community Composition:

The fact that the average species diversity beneath live hemlocks is lower than that beneath live birches, both are lower than that beneath dead hemlocks, and gaps have the highest species diversity suggests that more light is correlated with greater species diversity. This may be the case since only a select number of species are shade-tolerant. However, there could also be greater species diversity under dead trees and in gaps

because there is not a tree competing with the herbaceous vegetation for water or nutrients. The slopes of the curves for birch and dead hemlocks indicate that at this scale, birches add species quicker with increasing area though they may have fewer species than dead hemlocks in any given square meter. This suggests that the herbaceous stratum is less uniform beneath birches than dead hemlocks or live hemlocks, which is consistent with the idea that hemlocks act as engineer species. If they strongly modify the conditions on the forest floor beneath them, it makes sense that these conditions would be more uniform than those beneath a tree like a birch that modifies its environment less strongly so the conditions there are likely to represent residual effects of many historic factors.

Differences between the “engineering” capabilities of hemlock and birch were revealed in the ordination analysis as well. In the plots, the “hemlock effect” persisted for several years after the death of a hemlock. This was seen in the lack of distinction between live hemlocks and recently dead hemlocks and the intermediate position of gaps made by hemlocks in ordination space. When hemlocks die from the effects of adelgids, they often remain standing and drop all of their needles almost at once—this doesn’t create soil disturbance like a tip-up mound, and in fact it adds a layer of thick acidic needles that may act like mulch. This could retard the transition from vegetation associated with live hemlocks to vegetation associated with gaps or other canopy species. The vegetation on birch plots, on the other hand, seemed to have very little to do with the identity of the canopy tree, as shown by the fact that birch plots were scattered in all parts of the ordination space. Vegetation on birch plots differed by branch but not by focal tree category, supporting the conclusion that site variables like history and environmental factors associated with drainages affect the herb stratum in these plots much more than the birch itself.

It is interesting that plots in Hogskin Branch tended to cluster so strongly. The reason for this is not entirely obvious, but it was noticed during the course of the study that Hogskin Branch was markedly different from the other two branches in that it had no rhododendron even though it was adjacent to Pond Drain which had a fair amount and Hunters Branch which had rhododendron in abundance. Many differences in the herb layer could be due to this rhododendron understory. Alternatively, the indicator group of species for Hogskin Branch seems to be made up of species that are mostly found on cooler sites. Though the map elevations for the plots in Hogskin Branch are not significantly different from those in the other branches, the north-facing orientation of the drainage may create a cooler microclimate there, or harsher winter conditions. Four of the sixteen temperature sensors were located in Hogskin Branch, but due to such a small sample size and variation caused by sun flecks, it was not possible with the data collected during the study to determine whether Hogskin Branch was cooler on average.

With respect to the indicator species identified for each cluster, there are several interesting things to note. One is that all the species indicative of the hemlock cluster were woody rather than herbaceous. It might be that tree seedlings are better at tolerating very acidic soil conditions (Marx 2005 showed that acidic debris from hemlocks provided protection against fungal diseases for some seedlings). It would be informative to

determine the age of the seedlings to see if they are older seedlings waiting to be released or if they are predominantly first year seedlings that have been dispersed there but aren't likely to survive. Also, it might be that woody seedlings are found preferentially in hemlock plots because they cannot meet their germination and establishment needs as well in plots where competition from the faster-growing herbs is higher (Graves, Peet and White 2006). A second notable trend was that the most distinct cluster of plots in the ordination space was formed from old gaps made by hardwoods. This cluster also had the highest number of indicator species and the highest species diversity. This is not surprising in that these plot should not have been affected at all by hemlock modifications to the environment, plus light is abundant. The list of indicator species for this group contains rare species (e.g. *Scutellaria saxatilis*) and narrow endemics (e.g. *Boykinia aconitifolia*) alongside invasive exotics (e.g. *Cerastium glomeratum*). For this reason, it would be useful to conduct further studies on whether exotic species are just now arriving in this community or whether they have persisted there in low numbers for a relatively long time, and whether or not removal of hemlocks will give them a new advantage. Narrow endemics and rare species in gaps may not do as well with increased competition from exotic species.

### **Environmental Variables**

The fact that the soil beneath hemlocks and birches differs significantly provides support for the hypothesis that the different kinds of trees create different microenvironments. The differences in the soil could be due to the quicker turnover time for birch leaves and the slower decomposition of hemlock needles which causes a build-up of more organic material. More organic material lowers the soil pH, and has a strong affect on cation exchange capacity. Understory plants have different needs regarding soil pH and nutrient content, so significant differences in these soil variables between tree species may be a cause of differences in the understory plant community beneath them. Significant differences in soil variables for different locations in Pond Drain show that canopy tree type is not the only factor affecting soil. Tree-type may have an influence on the scale of tens of meters but this interacts with the influence of differences on a larger scale (e.g., site history, parent rock material, slope). Soil heterogeneity at this larger scale may explain the effect of stream branch on vegetation composition.

The relatively small variance in minimum daily temperature measurements compared to the large variance in maximum daily temperature measurements is probably due to the effect of sun fleck heterogeneity in locations where sensors were placed since they only took measurements at a single point. Direct sunlight could dramatically warm sensors during the day but would not affect sensors at night, so minimum temperatures at night are probably a better indicator of temperature differences between focal tree types because the temperature at the sensor is likely to be the same as the temperature over a whole plot. Better estimates of differences between maximum temperatures in the plots could be gained with a bigger sample size using more sensors or a temperature gun. The result showing a significant difference in minimum daily temperatures may indicate live hemlocks offer some degree of insulation and perhaps protection from frost. It would be interesting to use the sensors over a longer time period, especially during the winter

season, to see if this is the case. In the summer season, one degree may not make much difference. Because I was unable to learn anything conclusive about variation of maximum temperatures between tree types, it is not clear whether the warmer minimum temperatures below live hemlocks are due to insulation from dense canopy or to an albedo effect from the dark needles. If live hemlocks also have higher maximum daily temperatures, it would suggest that an albedo effect is a likely explanation, while more moderate live hemlock temperatures in general suggest the effects are due to insulation.

### **Implications**

Over the course of the study, it became clear that the study area is a remnant of intact old-growth forest, now very rare on the East Coast. Several species of uncommon plants occur in abundance in this habitat and there are hemlocks upwards of 400 years old (Wilbur, pers. com.). Some of these large hemlocks have survived the first wave of the Hemlock Woolly Adelgid and healthily persist, but many have died. It is unclear what factors led to the quick death of many hemlocks, caused intermediate symptoms in others, and left some apparently unaffected. Because of this, we do not know if the current die-off has peaked or whether the forest will continue to lose more and more trees. There is a lot of room for further investigation on this front. However, enough trees have already died completely that the character of the forest community will inevitably be changed to some degree. In addition to descriptions of current changes and differences, studies such as this one produce important baseline data that can be used to track changes in the forest-floor community, which may occur over a long period of time as areas beneath hemlocks become more exposed.

Analysis from this study suggests that there are differences in environmental variables and vegetation beneath live hemlocks, dead hemlocks, the gaps they eventually become, and the birches that may replace them. Because of this, it can be expected that old-growth hemlock forests that may have been unaltered for hundreds of years are on the brink of major change. This is an important example of the indirect affects of human activity on a system since both the Hemlock Woolly Adelgid and the exotic weeds that could potentially invade what is now a relatively pristine system were unintentionally introduced by humans. The infestation of hemlocks by adelgids also provides a good opportunity to examine one of the key issues in community ecology— in what ways can a single species control local biodiversity and what happens with the loss of a species that has strong transforming effects on its environment? This issue has relevance for other cases where a particular canopy tree species strongly influences understory composition and then declines, be it as a consequence of tree disease, global climate change, or any other factor. The unfolding of this story in one of our few remaining old-growth forests promises to be a very revealing and important process warranting future study.

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