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## CHANGING LITTER RESOURCES ASSOCIATED WITH HEMLOCK WOOLLY ADELGID INVASION AFFECT BENTHIC COMMUNITIES IN HEADWATER STREAMS

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CHANGING LITTER RESOURCES ASSOCIATED  
WITH HEMLOCK WOOLLY ADELGID INVASION  
AFFECT BENTHIC COMMUNITIES IN HEADWATER STREAMS

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THESIS

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A thesis submitted in partial fulfillment of the  
requirements for the degree of Master of Science in the  
College of Agriculture, Food and Environment  
at the University of Kentucky

By

Christopher J. Strohm

Lexington, Kentucky

Director: Dr. Lynne Rieske-Kinney, Professor of Entomology

Lexington, Kentucky

2014

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## ABSTRACT OF THESIS

### CHANGING LITTER RESOURCES ASSOCIATED WITH HEMLOCK WOOLLY ADELGID INVASION AFFECT BENTHIC COMMUNITIES IN HEADWATER STREAMS

Hemlock woolly adelgid is an invasive herbivore causing extensive mortality of eastern hemlock, an important foundation species that provides stable conditions influencing biological communities. Hemlock is often found in riparian areas and following its decline, broadleaved species, including birch, beech, and rhododendron, will replace it. These plants differ from hemlock in patterns of canopy cover and leaf properties, which influence conditions and resources within streams.

My goal was to evaluate potential impacts of adelgid-induced alterations to riparian canopies and litter on benthic communities and litter breakdown in streams. I characterized benthic invertebrate communities, litter colonization and litter breakdown in streams with hemlock- or deciduous-dominated riparian canopies. Riparian canopy influenced abundance of some invertebrates, but litter species influences a range of benthic colonizers. Rhododendron and beech litter generally support more invertebrates and decomposes more slowly than birch or hemlock. When invertebrates are excluded, broadleaved litter breakdown is more hindered than hemlock breakdown. My findings suggest that invertebrates may be more affected by future increases in broadleaved litter inputs to streams than by hemlock litter loss. This is significant because benthic invertebrates are important for in-stream litter processing and are linked to aquatic and terrestrial food webs.

Keywords: *Tsuga canadensis*, *Adelges tsugae*, riparian canopy, leaf decomposition, benthic invertebrates

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Christopher J. Stroh

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April 11, 2014

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## Table of Contents

Acknowledgements.....	iii
Table of Contents.....	v
List of Tables.....	vii
List of Figures.....	viii
Chapter 1:.....	1
Introduction.....	1
Chapter 2:.....	5
Shifting riparian canopy and litter inputs affect benthic invertebrate communities.....	5
Introduction.....	5
Materials & Methods.....	8
Study Sites.....	9
Litter Colonization.....	9
Litter Breakdown.....	11
Resident Invertebrates.....	11
Stream Characteristics.....	12
Analysis.....	12
Results.....	13
Litter Colonization.....	13
Litter Breakdown.....	16
Resident Invertebrates.....	16
Discussion.....	18
Litter Colonization.....	18
Litter Breakdown.....	22
Resident Invertebrates.....	23
Conclusions.....	24
Chapter 3:.....	37
Effects of invertebrate exclusion on litter breakdown.....	37
in Appalachian streams threatened by hemlock decline.....	37
Introduction.....	37
Materials & Methods.....	40



Study Site.....	40
Litter Bags .....	41
Litter Colonization.....	42
Litter Breakdown.....	42
Analysis .....	43
Results .....	44
Litter Colonization.....	44
Litter Breakdown.....	45
Discussion .....	46
Litter Colonization.....	46
Litter Breakdown.....	46
Conclusions .....	48
Appendices.....	56
Appendix 2.1. Characteristics of headwater streams with hemlock-dominated and deciduous-dominated canopies in southeastern Kentucky (Kentucky Ridge State Forest).....	56
Appendix 2.2. Total abundance of invertebrate families colonizing leaf litter in headwater streams with hemlock-dominated and deciduous-dominated canopies in central Appalachia over one year. ....	60
Appendix 2.3. Total abundance of invertebrate families collected in kick-net samples from headwater streams with hemlock-dominated and deciduous-dominated canopies in central Appalachia over one year. ....	64
Appendix 3.1. Analysis of microbial colonization.....	71
Appendix 3.2. Total abundance of invertebrate families colonizing leaf litter exposed in open and exclusion mesh in headwater streams with hemlock-dominated and deciduous-dominated canopies in central Appalachia. ....	73
References.....	76
Vita.....	84

## List of Tables

Table 2.1. Parameters (mean $\pm$ s.e.) of the colonizing invertebrate community based on a) riparian canopy, b) litter species, and c) season, in headwater streams in central Appalachia over one year.....	26
Table 2.2. Abundance (mean $\pm$ s.e.) of the most prevalent invertebrate families colonizing leaf litter in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year.....	28
Table 2.3. Abundance (mean $\pm$ s.e.) of colonizing invertebrate functional feeding groups in headwater streams based on a) riparian canopy, b) litter species, and c) season, in central Appalachia over one year.....	29
Table 2.4. Initial quality measurements (mean ( $\pm$ s.e.)) of leaves enclosed in mesh bags in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year. ....	31
Table 2.5. Community parameters of resident invertebrates (mean $\pm$ s.e.) sampled via a) kick-nets, and b) leaf-packs from headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year.....	32
Table 3.1. Abundance (mean $\pm$ s.e.) of all invertebrates, shredders, collector-gatherers, Chironomidae, and Leuctridae colonizing leaf litter exposed in open and exclusion bags in headwater streams of central Appalachia from winter to spring.....	50

## List of Figures

- Figure 2.1. Seasonal composition of functional feeding groups, including shredders, collector-gatherers, collector-filterers, and predators, colonizing litter of four species in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia. A.) mean number of invertebrates, and B.) percent of total .....34
- Figure 2.2. Percent of mass remaining (mean  $\pm$  s.e.) in mesh bags containing birch, beech, rhododendron, and hemlock leaves placed in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year.....35
- Figure 2.3. Seasonal abundance (mean  $\pm$  s.e.) of selected invertebrate families colonizing leaf litter in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia. A.) Hydropsychidae, B.) Chironomidae, C.) Leuctridae, and D.) Peltoperlidae.....36
- Figure 3.1. Invertebrate colonization ((mean ( $\pm$  s.e.) number of invertebrates) of leaf litter exposed in open and exclusion bags in headwater streams in central Appalachia from fall to spring.....51
- Figure 3.2. Benthic shredder colonization of four species of leaves in open and exclusion bags in headwater streams in central Appalachia.....52
- Figure 3.3. Litter breakdown of four tree species expressed as % mass remaining in open (dashed lines) and exclusion (solid lines) bags placed in headwater streams in central Appalachia in fall (0 d), winter (102 d), and spring (165 d).....53
- Figure 3.4. Percent of birch, beech, rhododendron, and hemlock leaf mass remaining in mesh bags in headwater streams in central Appalachia in fall (0 d), winter (102 d), and spring (165 d).....54
- Figure 3.5. Percent of litter mass remaining in exclusion and open treatment bags placed in headwater streams in central Appalachia in fall (0 d), winter (102 d), and spring (165 d). A. Birch, B. Beech, C. Rhododendron, and D. Hemlock .....55

## Chapter 1:

### Introduction

Eastern hemlock, *Tsuga canadensis* (L.) Carrière, is a foundation species in eastern North America that plays a significant role structuring forest communities (Ellison et al. 2005). A slow-growing and shade-tolerant tree (Godman and Lancaster 1990), hemlock represents important habitat for mammals, birds, and arthropods (Tingley et al. 2002, Rohr et al. 2009, Mallis and Rieske 2011, Sackett et al. 2011, Adkins and Rieske 2013, Johnson et al. *in press*). In the Appalachian region of North America, hemlocks are common and abundant alongside streams, and help stabilize thermal and hydrological regimes (Snyder et al. 2002), which influences fish (Ross et al. 2003) and benthic invertebrate communities (Snyder et al. 2002, Adkins 2012). Riparian hemlocks also contribute significant litter input into streams. Despite poor nutritional quality (Webster and Benfield 1986, Maloney and Lamberti 1995) and low retention in streams due to its size, hemlock needles are a prevalent resource because of hemlock's abundance near streams and its litterfall phenology (Adkins and Rieske *in review*).

Hemlock is currently experiencing widespread decline due to an exotic herbivore, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). Originally reported in eastern North America in Virginia in 1951, HWA is now established in the majority of hemlock's range (USDA Forest Service 2013). The adelgid feeds on xylem ray parenchyma cells (Young et al. 1995), depleting starch reserves (McClure 1991), inhibiting photosynthesis (Nelson et al. 2014), and causing eventual tree mortality. The extreme susceptibility of eastern hemlock to adelgid feeding

(Montgomery et al. 2009) and the lack of effective natural enemies for population regulation (Wallace and Hain 2000), has led to widespread hemlock mortality and extensive hemlock loss in riparian zones (Evans et al. 2011).

Co-occurring broadleaved trees and shrubs such as sweet birch (*Betula lenta* L.), American beech (*Fagus grandifolia* Ehrhart), and rhododendron (*Rhododendron maximum* L.) will be the replacement for dying hemlocks in the Appalachians (Spaulding and Rieske 2010, Krapfl et al. 2011, Ford et al. 2012). This plant community shift has special significance for headwater stream riparian areas because low order streams represent the maximum interface with surrounding vegetation (Vannote et al. 1980), deriving a large amount of energy and habitat conditions from nearby plants (Gregory et al. 1991). Hemlock's suite of characteristics, especially dense shading and persistent production of poor quality, slow-decomposing litter, make it a unique component of stream riparian corridors. The future plant community differs; sweet birch is an early-successional, shade-intolerant species (Burns and Honkala 1990) with leaves that abscise abruptly in the fall and form a large pulse of litter. Beech is a late-successional, shade-tolerant species (Burns and Honkala 1990) with some leaves abscising in fall and others being retained through winter. Both birch and beech are deciduous and have leaves with a rate of breakdown classified as 'medium' (Webster and Benfield 1986). Rhododendron is an ericaceous, evergreen understory shrub with most leaves abscising in fall (Monk et al. 1985). Leaf breakdown rate for rhododendron is classified as 'slow' (Webster and Benfield 1986).

Adelgid-induced decline of eastern hemlock and replacement by birch, beech, and rhododendron will alter the phenology, quantity, and composition of litter inputs entering

streams. This compositional shift will have effects on in-stream litter processing, which relies on benthic consumers. Litterfall phenology and initial leaf quality, which is a function of plant structural components, nutrients, and defensive compounds (Webster and Benfield 1986), largely defines the processing rate and benthic community associated with litter. Since birch, beech, and rhododendron leaves are of higher quality and have distinctly different physical and chemical characteristics from hemlock, in-stream litter processing dynamics will be different after hemlock loss. This change has vast implications, since litter which enters and is processed in streams becomes coarse and fine particulate organic matter (CPOM and FPOM) as well as dissolved organic matter (DOM) that deliver energy to many organisms downstream (Wallace et al. 1997).

The overall goal of my study is to evaluate potential impacts of altered riparian canopies and litter resources on benthic communities and litter decomposition. In chapter 2, I characterized the benthic invertebrate community colonizing litter of birch, beech, rhododendron, and hemlock, and subsequent breakdown in headwater streams with hemlock-dominated and deciduous-dominated canopies. Using mesh bags to enclose a known mass of litter, I monitored seasonal and species-specific colonization and breakdown. I also evaluated the same streams for resident invertebrates via kick-net and leaf-pack sampling, and physical and chemical characteristics, via stream water sampling. In chapter 3, I manipulated invertebrate and microbial colonization of birch, beech, rhododendron, and hemlock litter to determine their relative contributions to litter decomposition. I enclosed litter in mesh bags with different sized openings to allow or exclude invertebrates and monitored litter breakdown. My findings suggest that loss of

hemlock litter may not have a strong impact on invertebrates in these streams, but increasing inputs from birch, beech, and rhododendron may.

Very few studies have investigated invertebrate colonization of hemlock litter (but see Maloney and Lamberti 1995) so a complete understanding of its role in headwater stream communities is lacking. By characterizing the benthic community and decomposition dynamics associated with hemlock litter, and also of its broadleaved associates, my work helps elucidate future changes in stream communities that represent an indirect result of hemlock woolly adelgid invasion and the imminent loss of hemlock forests. Documenting these effects is important for gaining an understanding of how HWA is altering ecosystems in North America.

## Chapter 2:

### **Shifting riparian canopy and litter inputs affect benthic invertebrate communities**

#### Introduction

Changes in forest composition have cascading effects on associated communities, especially in riparian areas adjacent to headwater streams. Headwater stream watersheds are relatively small in area, and so are easily influenced by small-scale changes or localized disturbances (Lowe and Likens 2005, Meyer et al. 2007). Benthic invertebrates within these streams rely on allochthonous energy input provided primarily by surrounding vegetation, primarily leaf litter (Wallace et al. 1997). Disturbances to riparian vegetation that qualitatively or quantitatively alter litter availability influence invertebrate communities through bottom-up effects on invertebrate consumers (Wallace et al. 1997, Yoshimura 2012).

Riparian litter is broken down through leaching, physical abrasion, and microbial and invertebrate activity. Invertebrates that initially break down coarse leaf material ('shredders') feed on coarse particulate organic matter (CPOM) pre-conditioned by microbes (Cummins et al. 1989). Microbial conditioning and shredder utilization is dependent on physical and chemical characteristics of the litter, including cuticle thickness, defensive chemicals, nutrient content, and structural components (Webster and Benfield 1986). Plant taxa vary in these characteristics, creating a spectrum of litter resource quality and availability for microbes and invertebrates. The prevailing paradigm predicts that most deciduous leaves decompose more rapidly than conifer leaves, which are typically resistant to decomposition (Webster and Benfield 1986). Degradation of



CPOM by shredders and microbes generates fine particulate organic matter (FPOM) which is utilized by another invertebrate feeding group, the collectors. Both collector-gatherers and collector-filterers rely on FPOM generated from CPOM by microbes and shredders (Cuffney et al. 1990).

In central and southern Appalachia, eastern hemlock, *Tsuga canadensis* (L.) Carrière, is an important component of headwater stream riparian forests, where it is often the only conifer occurring. Hemlock is considered a foundation species in eastern North America (Ellison et al. 2005), where it represents crucial habitat for birds and arthropods (Tingley et al. 2002, Rohr et al. 2009, Mallis and Rieske 2011, Sackett et al. 2011, Adkins and Rieske 2013, Johnson et al. *in press*) and plays a significant role in structuring forest communities. Slow-growing and shade-tolerant (Godman and Lancaster 1990), hemlock's presence alongside streams can stabilize thermal and hydrological regimes and influence fish (Ross et al. 2003) and benthic invertebrate communities (Snyder et al. 2002, Adkins 2012). In addition, hemlock litter is a valuable resource for benthic invertebrates. Despite low retention in streams due to its size, hemlock needles are prevalent because of hemlock's abundance near streams and because of its relatively constant litterfall phenology (Adkins and Rieske *in review*).

As a coniferous member of the Pinaceae, hemlock litter breakdown is classified as 'slow', whereas co-occurring deciduous trees have litter classified as 'medium' or 'fast' (Webster and Benfield 1986). 'Slow' litter reaches peak microbial conditioning later in the season than 'fast' litter, thus it becomes suitable substrate for invertebrate colonization later, and supports different invertebrates than fast-decomposing litter (Petersen and Cummins 1974). This can lead to differences in benthic invertebrate

community structure when comparing communities near conifer-dominated forests to hardwood-associated communities (Hisabae et al. 2010).

In eastern North America eastern hemlock is currently threatened by an exotic insect herbivore, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). Native to Asia, hemlock woolly adelgid was first observed in eastern North America in Virginia in 1951. Wind, wildlife, and humans have aided in the spread of the adelgid (McClure 1990) to more than half of hemlock's range (US Forest Service 2013). The adelgid feeds on xylem ray parenchyma cells of all *Tsuga* species (Young et al. 1995), of which eastern hemlock is most susceptible (Montgomery et al. 2009). Adelgid feeding decreases eastern hemlock photosynthesis (Nelson et al. 2014), depletes starch reserves and significantly reduces growth, leading to rapid mortality (McClure 1991). In eastern North America native predators are unable to effectively regulate adelgid populations (Wallace and Hain 2000), and hemlock decline is occurring throughout its range (Evans et al. 2011).

If left unmitigated in the central and southern Appalachians, the adelgid is predicted to cause the near loss of hemlock-dominated forests, to be replaced by hardwood species (Spaulding and Rieske 2010, Ford et al. 2012). In riparian areas of this region, sweet birch (*Betula lenta* L.), American beech (*Fagus grandifolia* Ehrhart), and great laurel (*Rhododendron maximum* L.) co-occur with hemlock and will be involved in its replacement (Spaulding and Rieske 2010, Krapfl et al. 2011, Ford et al. 2012, Adkins and Rieske 2013). Sweet birch is an early-successional, shade-intolerant species (Burns and Honkala 1990) with leaves that abscise abruptly in fall. Beech is late-successional and shade-tolerant (Burns and Honkala 1990) with some leaves abscising in fall and

some retained through winter. Both birch and beech have leaves with a breakdown classified as ‘medium’ (Webster and Benfield 1986). In contrast, rhododendron is an ericaceous, evergreen understory shrub with most leaves abscising in fall (Monk et al. 1985), and a leaf breakdown rate classified as ‘slow’ (Webster and Benfield 1986).

Adelgid-induced decline of eastern hemlock and replacement by birch, beech, rhododendron, and similar broad-leaved species, will alter the composition of litter inputs entering streams. If the prevailing paradigm holds (Webster and Benfield 1986), this shift in the composition of litter inputs will likely have bottom-up effects on the invertebrate communities utilizing these resources. To determine the extent of these consequences in the wake of adelgid-induced shifts in forest composition, I evaluated benthic invertebrate colonization and breakdown of eastern hemlock, sweet birch, American beech, and rhododendron litter in streams with hemlock-dominated and deciduous-dominated riparian canopies. Few studies have evaluated benthic invertebrate colonization of eastern hemlock litter (Maloney and Lamberti 1995) and none have done so in the context of eastern hemlock decline. My overall goal was to understand the potential consequences of hemlock woolly adelgid-induced hemlock mortality and associated changes in forest composition on benthic invertebrate communities.

### Materials & Methods

I assessed benthic invertebrate colonization of leaf litter from eastern hemlock, sweet birch, American beech, and rhododendron. The resident benthic invertebrate community was assessed concurrently to evaluate the colonization potential of invertebrates present. Stream characteristics were also evaluated to determine the extent to which they relate to litter colonization and riparian canopy vegetation.

### *Study Sites*

Study sites were selected at Kentucky Ridge State Forest (Bell Co.), located in the Central Appalachian ecoregion of Kentucky and characterized by mixed mesophytic forests on steep hills and mountains underlain by Pennsylvanian shale, siltstone, sandstone, and coal. Streams are typically cool with moderate to high slopes and with cobble or boulder substrate (Woods et al. 2002). Hemlock woolly adelgid was initially reported in Bell County in 2007. Although it occurs sporadically throughout the forest, no hemlock decline was noted at my study streams.

Six first and second order perennial streams were selected based on similarities in watershed characteristics (Adkins 2012, Adkins and Rieske 2013). Vegetation surveys using USDA Forest Service common stand exam protocols (USDA 2009) were used to characterize riparian vegetation in 2008 (Adkins and Rieske 2013). Overstory hemlock stem density was used to designate three streams as ‘hemlock-dominated’ and three streams as ‘deciduous-dominated’ (Adkins and Rieske 2013). At each stream three sampling points were established within a 30 m reach to evaluate litter colonization, resident benthic invertebrates, and stream characteristics once during winter (March 30), spring (May 31), summer (July 31), and fall (September 26).

### *Litter Colonization*

To evaluate benthic invertebrate colonization, newly abscised leaves of sweet birch, American beech, and rhododendron were collected from the forest floor adjacent to each stream in November 2011; leaves of eastern hemlock were collected concurrently by clipping 3-5 cm twigs directly from trees. Leaves were returned to the laboratory and air dried. To evaluate initial carbon (C) and nitrogen (N) available to colonizing

invertebrates, subsamples of source leaf material were oven-dried at 60 °C, ground using a Wiley Mill, and injected into a LECO combustion instrument (LECO Inc., St. Joseph, MI). Percent C was calculated from the amount of organic material combusted and % N was measured via the amount of N<sub>2</sub> gas emitted during combustion.

Mesh bags (15.3 × 25.4 cm<sup>2</sup> with 2.5 mm<sup>2</sup> mesh size) were constructed of fiberglass (Gutter Guard, New York Wire Company, Hanover, PA, USA), filled with 5 g of air-dried leaves of each species, and sealed with a heated soldering gun. Each litter bag contained only a single species. At each of the three sampling points within each 30 m stream reach, four 0.6 m lengths of 12 mm rebar were anchored in the streambed, and one mesh bag of each litter type was randomly attached at 0.5 m intervals along the length of a steel cable. One cable (2.3 mm × 2.13 m) with mesh bags was attached to each embedded rebar and weighted down to ensure that all bags were at least partially submerged. Thus there were a total of 288 mesh bags, 72 of each litter species; 144 in streams with hemlock riparian canopy and 144 in streams with deciduous riparian canopy. Bags were deployed on 13 December 2011 and collected at 108, 170, 231, and 288 days (March 30, May 31, July 31, and Sept. 26, 2012). At each sample interval one cable containing a mesh bag of each litter species was removed. Mesh bags were sealed individually in 3.7 L plastic bags containing ~100 ml 70% ethanol, returned to the laboratory, and held at 4 °C until processing. In the laboratory invertebrates were separated from leaf material, counted, identified to family and assigned to functional feeding groups, including collector – filterers, collector – gatherers, predators, scrapers, and shredders (Merritt et al. 2008).

### *Litter Breakdown*

Prior to deployment ten 5 g subsamples of each species was retained, air-dried for 3 d, dried at 60 °C for 4 d, and weighed to calculate the approximate oven-dried mass contained within each litter bag. After collecting leaf material was air-dried for 3-6 d, oven-dried at 60 °C for 4 d, and weighed to the nearest 0.1 g to calculate dry mass remaining. Sub-samples (1 g ground leaves) were combusted at 500 °C for 5 hours to calculate the ratio of organic to inorganic matter and generate ash-free dry mass remaining. The natural logarithm of sample % mass remaining was regressed against time (days) to estimate the rate of decay ( $k$ ) for each litter species at each transect using the exponential decay model  $M_t = M_0 e^{-k t}$ , where  $M_t$  is the mass remaining at time  $t$  (days), and  $M_0$  is the mass remaining at time zero (Bärlocher 2005).

### *Resident Invertebrates*

To evaluate the resident invertebrate community available to colonize litter bags, kick-net samples and natural leaf-packs were collected at each sample interval. A standard kick-net sample (0.5 m<sup>2</sup> with a 30 sec kick interval) was taken ~10 m up/down stream from each sample point within a fast-moving (riffle) section of stream. Sample contents were transferred to a 710 mL plastic bag containing 70% ethanol, returned to the laboratory, and stored at 4 °C until processing.

Leaf-packs were collected concurrently to further characterize resident invertebrates and consisted of a handful of leaf and twig material (approximately 4 × 4 × 8 cm<sup>3</sup>) taken from natural accumulations in the stream that were within 1 m of litter bags. Leaf-pack samples were placed in 3.7 L plastic bags with ~100 mL 70% ethanol, sealed,

returned to the laboratory, and stored at 4 °C until processing. Resident invertebrates were processed as described above.

### *Stream Characteristics*

Stream characteristics were evaluated concurrently with invertebrate sampling (see Appendix 2.1).

### *Analysis*

Invertebrate family diversity was calculated using both Shannon's and Simpson's diversity indices ( $H = -\sum p_i \log p_i$ , where  $p_i$  = relative abundance of a given family, and  $D = 1 / [\sum (n / N)^2]$ , where  $n$  = total number of individuals in a given family,  $N$  = total number of individuals, respectively) (Magurran 2004). Invertebrate family evenness was calculated using Shannon's evenness measure ( $J' = H' / H_{\max}$ ), where  $H' = \ln(S)$ ,  $S$  = family richness, and  $H_{\max}$  = the maximum diversity possible in a sample. Similarity between invertebrate communities was quantified using the Sørensen coefficient of similarity ( $C_s = 2j / (a + b)$ ) where  $a$  = number of families present in community A,  $b$  = number of families present in community B and  $j$  = number of families in common between communities A and B (Southwood 1978). This coefficient of similarity was used to compare the colonizing invertebrate families to resident invertebrate families.

Invertebrate abundance, family richness, diversity (Shannon's and Simpson's indices), and evenness was compared across riparian canopies (hemlock versus deciduous), season (winter, spring, summer, and fall), and in the case of litter colonization, litter species (birch, beech, hemlock or rhododendron). These measures

were tested for normality using the Shapiro-Wilk's test (Shapiro and Wilk 1965) and, if needed,  $\sqrt{n} + 0.5$  transformed to meet normality assumptions.

A three-way repeated measures analysis of variance (ANOVA, PROC GLM, SAS v.9.1.3) with a split-plot design was used to analyze differences in litter colonization. Season was the repeated measure, riparian canopy was the whole plot and litter species was the split plot. Abundance, richness and diversity were the dependent variables analyzed. Initial litter quality was analyzed using a one-way ANOVA by species. The decay coefficient ( $k$ ) for litter was analyzed using a two-way ANOVA, with leaf species and canopy as main effects. A two-way ANOVA with riparian canopy and season as main effects was used to analyze differences in the resident invertebrate community. The difference of least squares was used to separate means for litter colonization, initial litter quality, litter breakdown, and resident invertebrates.

## Results

### *Litter Colonization*

Of the 9,660 colonizing invertebrates belonging to 51 families and ten orders, 47% (4,544) were collected from deciduous-dominated streams and 53% (5,116) were collected from hemlock-dominated streams (Appendix 2.2). Riparian canopy vegetation had no influence on any of the colonizing invertebrate population parameters I measured, but litter species and season affected several parameters (Table 2.1.b and c).

Abundance, family richness, and Shannon's diversity index were greater for invertebrates colonizing beech and rhododendron compared to those colonizing birch and hemlock (Table 2.1.b). Seasonal differences in family richness, diversity (Simpson's and



Shannon's), and evenness were apparent; all were greatest in winter (Table 2.1.c). There were no interactions between the main effects for any of the parameters evaluated.

Numerically dominant invertebrate families colonizing litter included Hydropsychidae (21%), Chironomidae (19%), Leuctridae (15%) and others (Table 2.2 and Appendix 2.2). Twenty-seven families colonized all of the four litter species; two families were unique to their litter species. Birch was colonized by 33 families. Beech litter was colonized by 33 families, with one (the Psychomiidae) being unique. Rhododendron was colonized by 39 families. Finally, hemlock was colonized by 33 families and Glossosomatidae was unique. The Sørensen coefficient of overlap for all litter comparisons equaled or exceeded 0.82 (range 0.82 – 0.92).

Riparian canopy had no effect on the abundance of the ten most prevalent families with the exception of Chironomidae ( $F_{1,4} = 4.47$ ,  $P < 0.05$ ) and Ephemerellidae ( $F_{1,4} = 10.95$ ,  $P < 0.05$ ), both of which were more abundant in hemlock-dominated streams (Table 2.2). Litter species affected colonization of the three most abundant invertebrate families. Hydropsychidae colonized rhododendron litter most ( $F_{3,12} = 30.16$ ,  $P < 0.01$ ), while Chironomidae colonized beech and rhododendron more readily than birch or hemlock ( $F_{3,12} = 16.74$ ,  $P < 0.01$ ). Leuctridae colonized beech litter more readily than the other litter species ( $F_{3,12} = 5.09$ ,  $P < 0.05$ ) (Table 2.2).

Of the functional feeding groups present, collector-gatherers and shredders dominated (35% each), followed by collector-filterers (21%), predators (9%), and scrapers (<1%). Riparian canopy influenced litter colonization only by collector-gatherers, which were more abundant in litter from hemlock-dominated streams (Table

2.3.a). Litter species and season affected all functional feeding groups evaluated (Tables 2.3.b and 2.3.c). There was a significant canopy  $\times$  season interaction for collector-filterers ( $F_{3,12} = 5.42$ ;  $P = 0.01$ ) but no interactions were significant for my other functional groups.

Shredder colonization was highest in winter, declining in spring and declining further in summer and fall (Table 2.3.c). Shredders colonized beech and rhododendron litter similarly, but only colonized beech more than birch and hemlock (Table 2.3.b). Collector-gatherer colonization was greatest in spring (Table 2.3.c), and occurred more readily in beech and rhododendron litter than birch or hemlock (Table 2.3.b). Collector-filterer colonization of my litter species was lowest in winter and spring (Table 2.3.c); colonization of hemlock and rhododendron litter was similar, but only colonization of rhododendron was greater than of birch and beech (Table 2.3.b). Predator colonization of litter was relatively similar across seasons but was greatest in summer and fall (Table 2.3.c). Predators colonized beech the most and hemlock the least (Table 2.3.b).

Within each season the relative composition of functional feeding groups colonizing my litter, regardless of species, shifted slightly (Fig. 2.1). Shredders dominated the colonizing community in winter, shifting to collector-gatherers in spring (Fig. 2.1), driven primarily by their abundance in beech litter (spring collector-gatherer means: beech =  $37.88 \pm 13.21$ ; rhododendron =  $17.06 \pm 4.24$ ; hemlock =  $9.5 \pm 2.94$ ; birch =  $7.56 \pm 1.51$ ). In summer collector-filterers became more abundant (Table 2.3.c), and this pattern persisted through the fall (Fig. 2.1).

### *Litter Breakdown*

Initial litter quality differed among species. Hemlock contained the greatest percent C and N but had the lowest C:N ratio, whereas rhododendron contained the lowest C and N, but had the highest C:N ratio (Table 2.4). Riparian canopy and litter species affected the rate of litter breakdown ( $F_{1,47} = 8.75$ ,  $P < 0.05$ , and  $F_{3,47} = 11.37$ ,  $P < 0.0001$ , respectively). Litter in hemlock-dominated streams had a faster rate of decay ( $k$ ) than litter in deciduous-dominated streams (hemlock:  $-0.0101 \pm 0.0005$  vs. deciduous:  $-0.0084 \pm 0.0004$ ). Among my litter species, birch decayed most rapidly ( $-0.0111 \pm 0.0006$ ), hemlock ( $-0.0097 \pm 0.0004$ ) and beech ( $-0.0090 \pm 0.0008$ ) were similar and slower, and rhododendron was slowest ( $-0.0062 \pm 0.0005$ ). The mass remaining (%) of beech and rhododendron litter was similar at each sample interval and was greater than hemlock or birch (Fig. 2.2).

### *Resident Invertebrates*

Kick-net sampling for resident invertebrates yielded 2,837 individuals; 1,012 (36%) from deciduous-dominated streams and 1,825 (64%) from hemlock-dominated streams. Forty-five families from ten orders were represented (Appendix 2.3), including the numerically dominant Chironomidae (16%), Hydropsychidae (16%), and Leuctridae (13%). Resident invertebrates consisted of collector-gatherers (32%), shredders (29%), collector-filterers (20%), predators (10%), and scrapers (9%).

In kick-net samples of resident invertebrates, riparian canopy, but not season, influenced invertebrate abundance ( $F_{1,63} = 7.65$ ,  $P < 0.01$ ), family richness ( $F_{1,63} = 8.82$ ,  $P < 0.01$ ), and Shannon's diversity index ( $F_{1,63} = 8.48$ ,  $P < 0.01$ ). In streams with a hemlock-dominated riparian canopy, abundance, family richness, and diversity

(Shannon's) of invertebrates was greater than in streams with a deciduous-dominated canopy (Table 5.a). Only collector-gatherer abundance was influenced by riparian canopy ( $F_{1,63} = 7.98, P < 0.01$ ) and was greater in hemlock-dominated streams (mean =  $17.4 \pm 2.98$  vs. deciduous-dominated mean =  $8.53 \pm 1.14$ ). In contrast, season did influence Simpson's diversity index ( $F_{1,63} = 6.64, P < 0.01$ ) and Shannon's evenness ( $F_{1,63} = 6.08, P < 0.01$ ). Both measures were lowest in fall (Table 2.5.a). There were no interactions between any of the main effects.

Resident invertebrates were also evaluated via leaf-pack sampling, which generated 2,761 invertebrates; 1,688 (61%) from deciduous-dominated streams and 1,073 (39%) from hemlock-dominated streams. Invertebrates from leaf-packs belonged to 35 families in ten orders (Appendix 2.4), including Peltoperlidae (25%), Leuctridae (14%), and Chironomidae (14%). Leaf-pack invertebrates were dominated by shredders (52%) and collector-gatherers (33%). Predators (9%), collector-filterers (4%), and scrapers (2%) were also present.

Riparian canopy alone did not influence population parameters of resident invertebrates sampled from leaf packs. There was a canopy  $\times$  season interaction ( $F_{3,64} = 2.87, P < 0.05$ ) for predators, with greater abundance in deciduous-dominated streams in summer and fall. Season influenced invertebrate abundance ( $F_{3,64} = 3.45, P < 0.02$ ), family richness ( $F_{3,63} = 6.02, P < 0.01$ ), and Shannon's diversity ( $F_{3,64} = 3.97, P < 0.01$ ). Invertebrates colonizing leaf-packs in spring were less abundant, with lower richness and less diversity (Shannon's) than in winter, summer, and fall (Table 2.5.b). Season also affected the abundance of collector-gatherers ( $F_{1,64} = 7.37, P < 0.001$ ) and scrapers ( $F_{1,64} = 9.40, P < 0.0001$ ), both were most abundant in fall (Table 2.5.b).

Total family richness of resident invertebrates sampled via leaf-packs was 34, compared to 41 of litter colonizing invertebrates. The Sørensen coefficient of overlap between the two was 0.88. No families were unique to the leaf-pack community and only one was unique to the colonizing community (Trichoptera: Molannidae). In comparing the leaf-pack community to the community colonizing each individual litter species, the Sørensen coefficient of overlap equaled or exceeded 0.87 and rhododendron was most similar (0.93).

## Discussion

I evaluated spatial and temporal differences in benthic invertebrate colonization of leaf litter in headwater streams with the goal of understanding how shifting forest composition due to hemlock woolly adelgid-induced eastern hemlock mortality may affect these communities. I document shifts in the benthic invertebrate community, affected by both riparian canopy and litter availability, which challenge the current paradigm of litter breakdown and the role of benthic invertebrates.

### *Litter Colonization*

I found riparian canopy and litter species are important determinants explaining differences in abundance and diversity among some colonizing invertebrates. Colonization of litter by collector-gatherers, primarily chironomid midge larvae and ephemereid mayfly nymphs, was greater in hemlock-dominated streams, corroborating findings in other regions. Willacker et al. (2009) report greater collector-gatherer abundance in a hemlock stream compared to a deciduous stream in New England, and several genera of Ephemereidae are reportedly weak associates of hemlock-dominated headwater streams in the mid-Atlantic (Snyder et al. 2002). Hydrologic and thermal

stability has been attributed to hemlock riparian canopies and provided as supporting evidence for the ability of these streams to support unique taxa (Snyder et al. 2002), but it's unlikely that either hydrology or temperature is causing the differences in abundance I found. Ephemerellidae are sensitive gill breathers that would certainly benefit from cooler streams with stable flow, but Chironomidae are some of the most resilient of aquatic animals (Merritt et al. 2008), and would likely be unaffected. Rather, qualitative and/ or quantitative differences in resources in the form of allochthonous inputs are more likely responsible for the greater ephemerellid and chironomid collector-gatherer abundance in hemlock-dominated streams that I report.

Litter quality likely plays a key role in the early stages of colonization. Initial nitrogen concentrations across species were highly variable; beech and rhododendron had lower concentrations than hemlock or birch. If nitrogen were the sole driver of benthic invertebrate colonization rates, I would expect shredders, the first colonizers, to most rapidly colonize higher nitrogen hemlock and birch litter. But benthic invertebrates utilizing these resources must contend with qualitative characteristics beyond simple nutrients. While hemlock contained high concentrations of nitrogen, it also had the lowest C:N ratio of any of the species evaluated, which undoubtedly influenced invertebrate utilization. Additionally, my first measure of invertebrate colonization was in winter, 108 d after deployment. That time interval would have allowed nitrogen to leach out, generating a more similar profile across species.

All litter species in my study were readily colonized, but a more abundant and taxa-rich invertebrate community colonized beech and rhododendron litter relative to birch and hemlock. I found that shredders more readily colonize higher quality litter such

as beech and rhododendron. Given the relatively rapid rate of hemlock litter breakdown in streams coupled with a lack of invertebrate colonization, it's clear that the role of microbial colonization needs further investigation. Colonization of hemlock litter by shredders, represented primarily by leuctrid and peltoperlid stoneflies (Table 2.2), was relatively low, in spite of their persistent occurrence in my samples (Fig. 2.3.c and d). Leuctridae have been reported numerically dominating the shredder community in hemlock streams in Appalachia (Adkins and Rieske *in review*). In the mid-Atlantic, Snyder et al. (2002) note that *Leuctra* nymphs are weakly associated with hemlock-dominated streams, and Rohr et al. (2009) found *L. ferruginea* adults to be an indicator species of hemlock canopies. My findings do not suggest a hemlock habitat or litter association for Leuctridae or other shredder families. Instead, my findings suggest that hemlock litter remains a relatively poor quality food resource, even after extended exposure in streams allows for microbial conditioning.

Collector-gatherers and collector-filterers also colonized beech and rhododendron more so than birch and hemlock, suggesting that beech and rhododendron represent a higher quality resource, not only to shredders feeding on CPOM, but also to FPOM-feeders, at least in the timeframe of my study (March to September of the year following abscission). My two most abundant families, Hydropsychidae (Trichoptera) and Chironomidae (Diptera), feed on FPOM. Hydropsychid caddisflies collect FPOM from silk-spun nets that they build inside retreats on submerged substrates (Merritt et al. 2008). I found them most abundantly on rhododendron litter, which likely provides a more suitable substrate for collecting FPOM due to the large leaf size and resilience to breakdown, relative to beech, birch, or hemlock. In contrast, chironomid midges are

ubiquitous, and burrow into substrate to collect FPOM (Merritt et al. 2008). In my study chironomids more readily colonized beech and rhododendron litter, perhaps due to the relatively slow rate of decomposition that provides a more stable substrate, and therefore more stable food source, relative to the other species available.

Beech and rhododendron leaves, whether as a food source, habitat substrate, or both, are a stable resource for benthic invertebrates. Rhododendron is expected to increase in abundance following adelgid-induced hemlock decline, and beech may be a part of the replacement forest where rhododendron does not limit its establishment (Spaulding and Rieske 2010, Ford et al. 2012). The increasing prevalence of beech and rhododendron across the affected region will certainly increase their litter inputs into streams. Increasing litter inputs can potentially increase production and thus population growth (Richardson 1991), whereas reducing litter inputs could limit stream invertebrate populations (Wallace et al. 1999). Thus if these insects are resource-limited, more beech and rhododendron litter in streams could increase the abundance of shredders, which in turn would enhance FPOM resources, leading to an increase abundance of collector-gatherers and collector-filterers.

Seasonal shifts in abundance and diversity of colonizing invertebrates were evident, as expected, due to life history traits and to shifting resource availability as physical, microbial, and shredder-associated litter breakdown progressed. Not surprisingly, winter litter colonization was dominated by shredders, the first invertebrate feeding group to process microbial-conditioned CPOM (Table 2.3.c and Fig. 2.1). By spring the collector-gatherers, which feed on shredder-produced FPOM, replace shredders as the dominant group, driven in part by chironomid midges, which increased



in abundance in spring (Table 2.3.c). In summer, collector-filterers, also feeding on shredder-produced FPOM, colonize litter in greater numbers (Table 2.3.c), partly driven by hydropsychid caddisflies (Fig. 2.3.a); this contributes to a more even distribution of functional feeding groups that persists into fall (Fig. 2.1).

### *Litter Breakdown*

Breakdown of all litter I evaluated was accelerated in streams where hemlock was the dominant overstory tree. This may be attributable to both biotic and abiotic factors. Collector-gatherers colonized litter in hemlock-dominated streams more readily than in deciduous-dominated streams. While collector-gatherers primarily utilize FPOM and are not essential to the initial stages of litter degradation, they do contribute to decomposition (Dieterich et al. 1997) and therefore could potentially increase litter decay. Although I did not measure stream flow, headwater streams with watersheds dominated by hemlock are noted for having stable hydrology (Snyder et al. 2002); more stable flow could accelerate litter breakdown rate via physical abrasion.

Low nitrogen leaves tend to break down more slowly than those with high nitrogen (Webster and Benfield 1986). I found that beech and rhododendron breakdown was slow, and also that they had initially low nitrogen concentrations. Hemlock had the highest initial nitrogen concentrations and its breakdown was relatively fast. The rapid degradation of hemlock I observed challenges the prevailing paradigm of pinaceous litter breakdown (Webster and Benfield 1986), which is considered somewhat resilient due to chemical and physical barriers. The rapid breakdown of hemlock may also be an artifact of leaf size. The narrow (<2mm) hemlock needles could work their way through the fiberglass mesh when bags were disturbed. So during periods of high stream flow

hemlock breakdown may have been accelerated by physical abrasion more than its broadleaved counterparts.

Nevertheless, in my study beech and rhododendron litter retained 15-35% of original mass through the summer (July 31) and fall (Sept. 26) sampling. This is in stark contrast to birch and hemlock, in which only 5-10% of original mass remained by Sept. 26 (Fig. 2.2). Less mass remaining equates to less substrate and/or food for invertebrates and subsequently less colonization later in the year. After hemlock decline and replacement, Appalachian streams will receive greater inputs from these more stable resources which persist as a detrital resource during summer and fall. Summer is a period when litter inputs may be lacking and can reduce invertebrate abundance in streams (Grubbs and Cummins 1994). Thus, beech and rhododendron litter may be especially important for invertebrates which are developing in summer.

#### *Resident Invertebrates*

Resident invertebrates collected via kick-net sampling were more abundant and diverse in hemlock-dominated streams than deciduous-dominated streams, but there were no riparian canopy differences in resident invertebrates sampled from leaf-packs. Within the kick-net community, only collector-gatherers were more abundant in hemlock-dominated streams and are undoubtedly the cause of this trend. Collector-gatherers constituted one third of the resident invertebrates and were primarily chironomid midge larvae. Only in kick-net sampling are riparian canopy-related differences apparent. This disparity is likely due to differences in the habitats sampled (riffles vs. pools). Riffle habitats support more collector-gatherers when hemlocks dominate the riparian canopy but leaf-pack habitats did not. Snyder et al. (2002) found that daily and seasonal

fluctuations in stream flow were lower in hemlock-dominated streams than in deciduous counterparts, and this could be occurring in my study streams. Benthic invertebrates in riffles would be exposed to greater changes in stream flow than invertebrates in naturally occurring leaf-packs congregating in low-flow areas or pools. If flow is less variable in hemlock-dominated streams, this may explain why abundance differed between riparian canopies in kick-net samples.

### *Conclusions*

Appalachian headwater streams with deciduous-dominated canopies and a lack of hemlock litter inputs are indicative of the future habitat and resources available to benthic invertebrates after hemlock woolly adelgid-induced hemlock decline. My results demonstrate that canopy hemlock loss may slow litter breakdown and will likely affect specific groups of stream invertebrates. But my results also suggest that changing litter inputs may strongly affect a wider range of invertebrates because of the importance of litter as a resource for both food and habitat of multiple functional groups. I found that hemlock litter is a low quality resource with low retention in streams and, in my study, relatively fast breakdown. Despite its poor qualities, hemlock litter is currently a major input source to these streams and processing must occur. Clearly the role of microbial colonization in breakdown of these litter species requires further study. The loss of hemlock litter inputs may be less important to benthic invertebrates than future increases in broadleaved litter inputs. In particular, my study shows that beech and rhododendron litter support higher densities of invertebrates and greater family-level diversity due to their longevity in streams.

These changes have consequences for both aquatic and terrestrial food webs. While immature aquatic insects form an important linkage in the stream food web, emergent winged adults leave the water and can be an important part of the terrestrial food web (Henschel et al. 2001). Thus, higher trophic levels could be affected by changes in the benthic invertebrate community.

Although I am gaining a more complete understanding of how benthic invertebrates utilize different litter sources, the full implications of the loss of hemlock litter after riparian hemlock decline remains unclear.

Tables and Figures

Table 2.1. Parameters (mean  $\pm$  s.e.) of the colonizing invertebrate community based on a) riparian canopy, b) litter species, and c) season, in headwater streams in central Appalachia over one year. Means with the same letter within each effect are not significantly different ( $P < 0.05$ ).

Effect		Abundance	Family richness	Shannon's diversity	Simpson's diversity	Evenness
a. Canopy	$F_{1,4} / P$	0.07 / 0.80	0.00 / 0.96	0.02 / 0.89	0.00 / 0.99	0.00 / 0.97
Hemlock		38.19 (4.12) a	5.95 (0.26) a	1.24 (0.05) a	0.65 (0.02) a	0.73 (0.02) a
Deciduous		32.22 (3.41) a	5.71 (0.25) a	1.23 (0.05) a	0.64 (0.02) a	0.71 (0.02) a
b. Litter species	$F_{3,12} / P$	7.60 / <0.01	4.53 / 0.02	3.75 / 0.04	3.27 / 0.06	2.79 / 0.09
Birch		30.93 (5.82) a	5.23 (0.37) a	1.12 (0.07) a	0.59 (0.03) a	0.67 (0.04) a
Beech		43.81 (5.26) b	6.56 (0.38) b	1.34 (0.06) b	0.68 (0.03) a	0.74 (0.03) a
Rhododendron		38.72 (5.09) b	6.25 (0.35) b	1.29 (0.06) b	0.66 (0.03) a	0.72 (0.03) a
Hemlock		27.37 (4.99) a	5.29 (0.31) a	1.19 (0.06) a	0.65 (0.03) a	0.74 (0.03) a
c. Season	$F_{3,12} / P$	0.65 / 0.60	6.10 / 0.01	12.54 / 0.001	14.36 / <0.01	13.24 / <0.01

Table 2.1 (continued)

Winter	29.97 (2.15) a	6.91 (0.29) b	1.53 (0.05) c	0.75 (0.02) b	0.81 (0.02) b
Spring	34.19 (4.76) a	5.11 (0.33) a	1.05 (0.06) a	0.55 (0.03) a	0.64 (0.03) a
Summer	37.01 (7.38) a	5.59 (0.37) a	1.21 (0.07) b	0.67 (0.03) ab	0.75 (0.03) b
Fall	39.62 (5.85) a	5.71 (0.40) a	1.16 (0.07) a	0.61 (0.03) a	0.67 (0.04) a

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Table 2.2. Abundance (mean  $\pm$  s.e.) of the most prevalent invertebrate families colonizing leaf litter in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year. Means with the same letter within each effect are not significantly different ( $P < 0.05$ ).

Effect	Canopy		Litter			
	Hemlock	Deciduous	Birch	Beech	Rhododendron	Hemlock
Hydropsychidae	8.73 (2.22) a	5.91 (1.80) a	7.67 (3.34) a	4.40 (1.38) a	9.47 (3.28) b	7.59 (2.93) a
Chironomidae	8.49 (1.58) b	4.76 (0.65) a	3.57 (0.67) a	8.94 (2.24) b	7.91 (1.44) b	5.96 (1.94) a
Leuctridae	4.15 (0.95) a	6.55 (1.30) a	6.07 (2.07) a	9.19 (1.92) b	3.88 (1.37) a	2.46 (0.74) a
Peltoperlidae	2.78 (0.42) a	2.52 (0.38) a	2.29 (0.52) ab	3.25 (0.67) b	3.50 (0.64) b	1.59 (0.36) a
Tomoceridae	1.27 (0.29) a	2.30 (0.39) a	1.59 (0.35) a	2.28 (0.72) a	2.06 (0.54) a	1.29 (0.24) a
Lepidostomatidae	2.13 (0.34) a	1.36 (0.23) a	1.39 (0.34) a	1.79 (0.36) a	2.10 (0.54) a	1.66 (0.38) a
Ephemerellidae	3.19 (0.48) b	0.29 (0.06) a	1.96 (0.58) a	1.25 (0.38) a	1.88 (0.57) a	1.71 (0.45) a
Nemouridae	1.06 (0.25) a	2.17 (0.44) a	1.55 (0.52) a	2.15 (0.63) a	1.21 (0.40) a	1.61 (0.48) a
Perlodidae	1.11 (0.22) a	1.52 (0.24) a	1.19 (0.34) a	1.32 (0.30) a	1.65 (0.39) a	1.14 (0.29) a
Isotomidae	1.96 (1.43) a	0.30 (0.11) a	0.49 (0.18) a	3.63 (2.82) b	0.24 (0.09) a	0.13 (0.06) a

Table 2.3. Abundance (mean  $\pm$  s.e.) of colonizing invertebrate functional feeding groups in headwater streams based on a) riparian canopy, b) litter species, and c) season, in central Appalachia over one year. Means with the same letter within each effect are not significantly different ( $P < 0.05$ ).

Effect		Shredders	Collector-Gatherers	Collector-Filterers	Predators
a. Canopy	$F_{1,4} / P$	0.08 / 0.79	14.79 / <0.01	2.45 / 0.13	1.92 / 0.24
Hemlock		10.49 (1.21) a	16.12 (2.28) b	8.78 (2.23) a	2.62 (0.40) a
Deciduous		13.74 (1.68) a	8.47 (0.79) a	6.15 (1.83) a	3.73 (0.44) a
b. Litter species	$F_{3,12} / P$	4.39 / 0.03	15.01 / <0.01	3.49 / 0.05	5.80 / 0.01
Birch		12.13 (2.59) a	8.33 (1.13) a	7.83 (3.36) a	2.52 (0.45) ab
Beech		17.10 (2.39) b	17.31 (3.85) b	4.56 (1.39) a	4.68 (0.75) c
Rhododendron		11.71 (1.88) ab	13.60 (1.57) b	9.62 (3.31) b	3.60 (0.71) bc
Hemlock		7.81 (1.11) a	9.69 (2.06) a	7.71 (2.97) ab	2.0 (0.37) a
c. Season	$F_{3,12} / P$	25.24 / 0.03	4.36 / 0.03	56.91 / <0.01	5.65 / 0.01
Winter		17.04 (1.51) c	7.03 (0.88)	3.57 (0.64)	2.17 (0.32)
Spring		13.44 (2.32) b	18.00 (3.77)	0.97 (0.60)	1.66 (0.25)



Table 2.3 (continued)

Summer	7.69 (1.53) a	11.47 (2.14)	13.91 (4.63)	3.82 (0.57)
Fall	10.24 (2.65) a	12.03 (1.31)	11.85 (3.26)	5.26 (0.96)

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Table 2.4. Initial quality measurements (mean ( $\pm$  s.e.)) of leaves enclosed in mesh bags in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year. Means with the same letter within each measure are not significantly different ( $P < 0.05$ ).

Species	% C	% N	C:N
	$F_{3,8} = 104.53 / P < 0.0001$	$F_{3,8} = 1601.06 / P < 0.0001$	$F_{3,8} = 1322.38 / P < 0.0001$
Birch	48.35 (0.10) b	1.01 (0.01) c	47.76 (0.54) b
Beech	47.89 (0.08) a	0.94 (0.001) b	51.00 (0.05) c
Rhododendron	47.72 (0.03) a	0.58 (0.01) a	81.88 (0.92) d
Hemlock	49.23 (0.04) c	1.40 (0.01) d	35.24 (0.19) a

Table 2.5. Community parameters of resident invertebrates (mean  $\pm$  s.e.) sampled via a) kick-nets, and b) leaf-packs from headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year. Means with the same letter within each effect are not significantly different ( $P < 0.05$ ).

Effect	Canopy		Season			
	Hemlock	Deciduous	Winter	Spring	Summer	Fall
a. Kick-net						
Abundance	52.14 (8.66) b	28.11 (3.99) a	38.56 (10.64) a	43.06 (11.04) a	25.94 (5.09) a	51.50 (10.64) a
Family Richness	11.51 (0.75) b	8.53 (0.62) a	10.06 (1.07) a	10.33 (0.72) a	8.94 (1.17) a	10.61 (1.12) a
Shannon's Diversity	1.99 (0.06) b	1.73 (0.07) a	1.94 (0.09) a	1.93 (0.06) a	1.78 (0.12) a	1.77 (0.11) a
Simpson's Diversity	0.85 (0.01) a	0.84 (0.02) a	0.88 (0.01) b	0.85 (0.01) b	0.89 (0.02) b	0.77 (0.04) a
Shannon's Evenness	0.85 (0.01) a	0.86 (0.02) a	0.89 (0.02) b	0.85 (0.02) ab	0.89 (0.02) b	0.79 (0.03) a
b. Leaf-pack						
Abundance	29.81 (4.44) a	46.89 (9.66) a	43.61 (7.79) b	18.22 (4.05) a	38.72 (12.19) b	52.83 (14.81) b
Family Richness	6.50 (0.51) a	6.92 (0.63) a	7.33 (0.75) b	4.28 (0.58) a	6.67 (0.70) b	8.56 (0.84) b
Shannon's Diversity	1.36 (0.08) a	1.38 (0.10) a	1.50 (0.09) b	1.01 (0.14) a	1.41 (0.13) b	1.54 (0.10) b

Table 2.5 (continued)

Simpson's Diversity	0.68 (0.03) a	0.69 (0.04) a	0.75 (0.03) a	0.58 (0.07) a	0.70 (0.06) a	0.72 (0.03) a
Shannon's Evenness	0.77 (0.03) a	0.74 (0.04) a	0.80 (0.03) a	0.68 (0.08) a	0.78 (0.05) a	0.75 (0.03) a

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Figure 2.1. Seasonal composition of functional feeding groups, including shredders (■), collector-gatherers (▨), collector-filterers (▩), and predators (▧), colonizing litter of four species in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia. A.) mean number of invertebrates, and B.) percent of total.

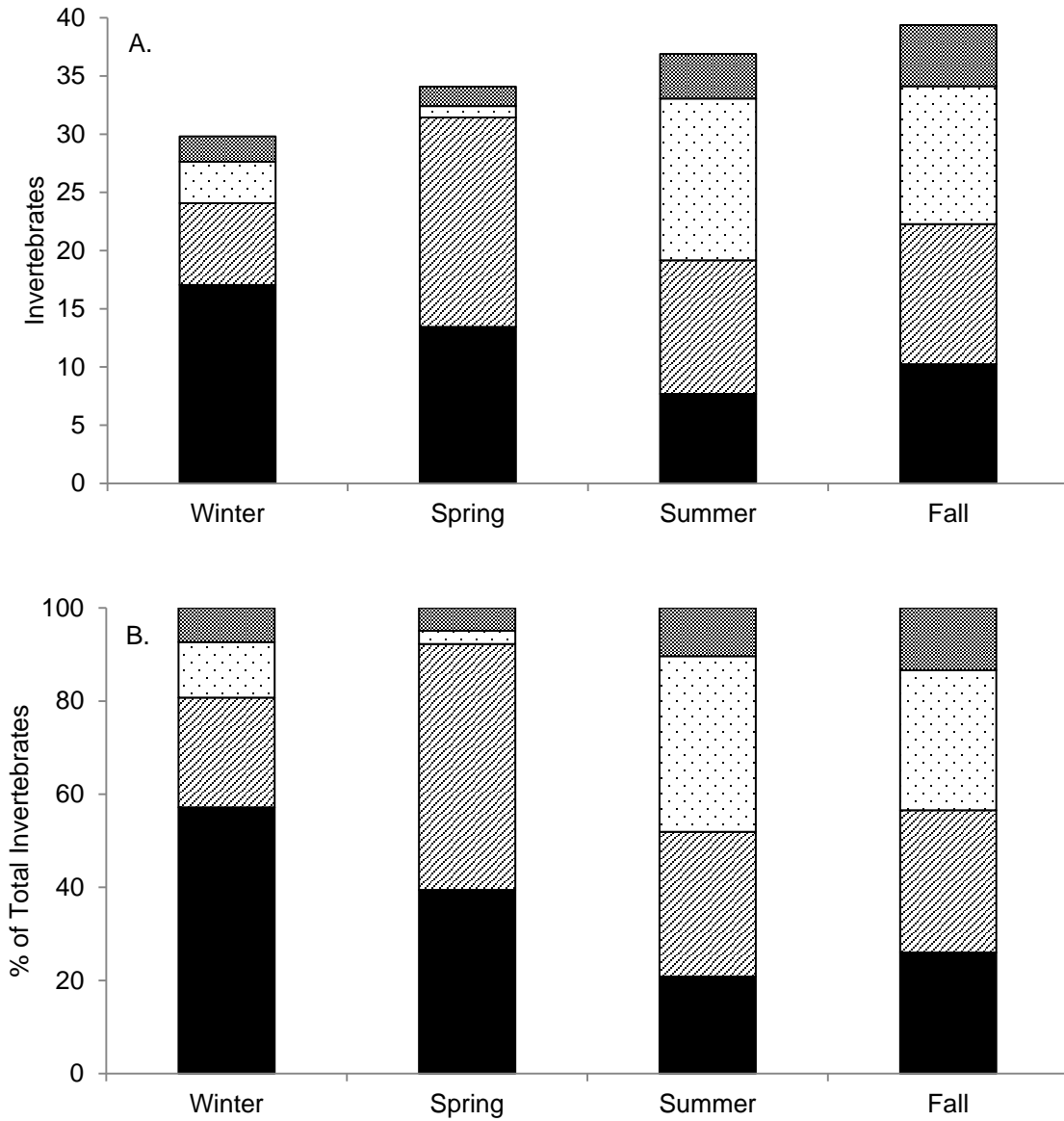


Figure 2.2. Percent of mass remaining (mean  $\pm$  s.e.) in mesh bags containing birch, beech, rhododendron, and hemlock leaves placed in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia over one year.

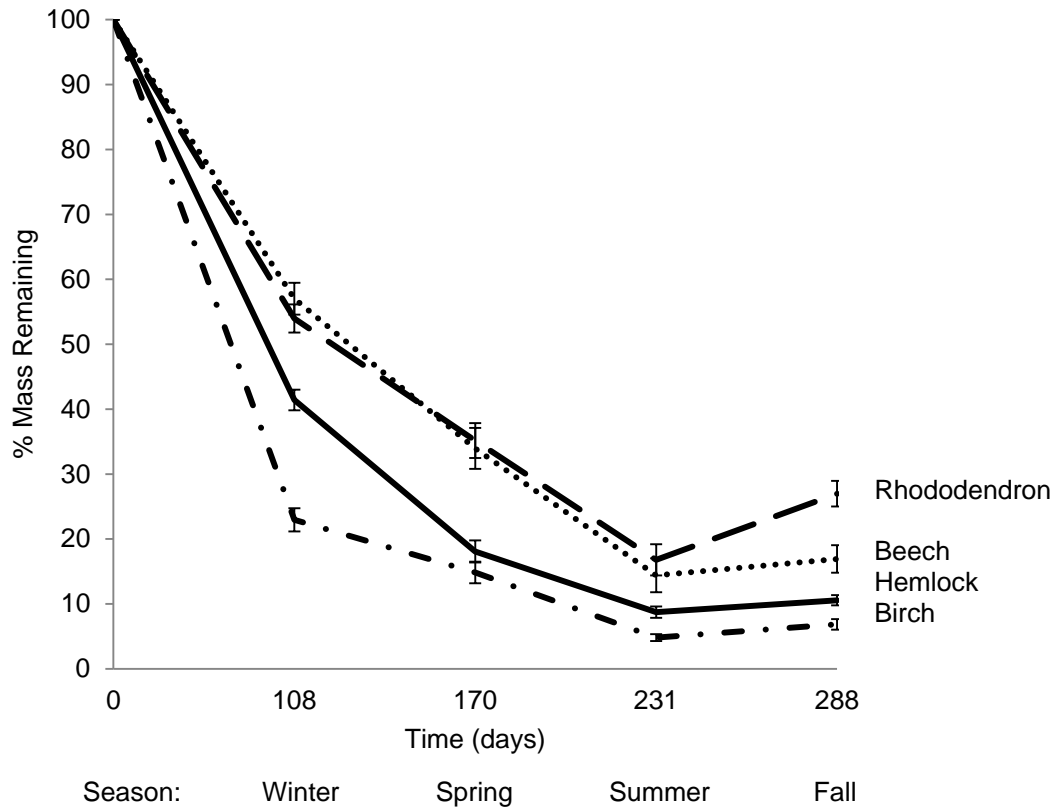
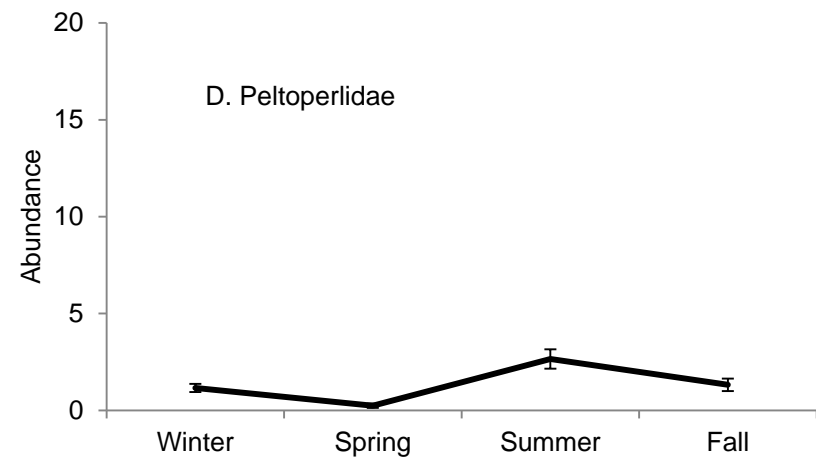
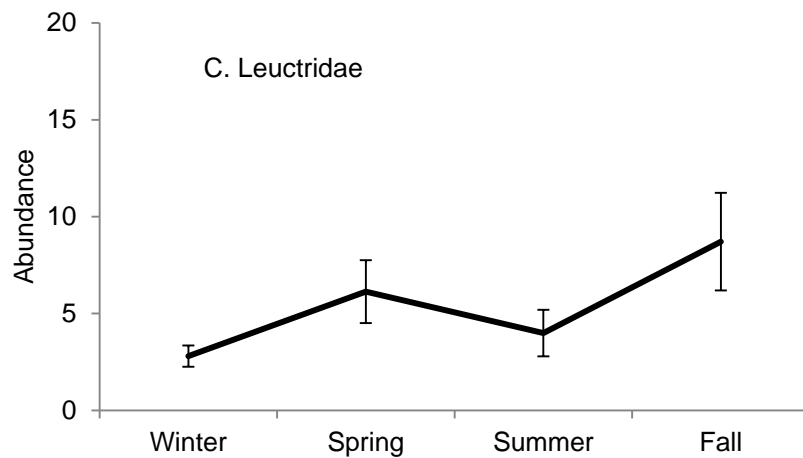
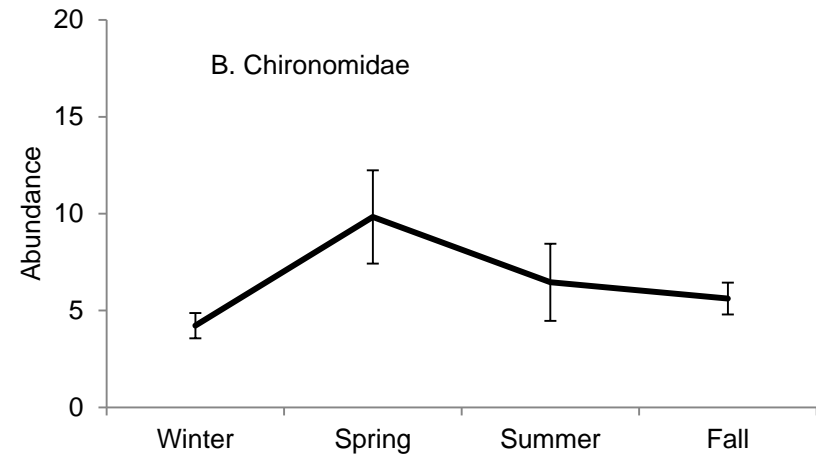
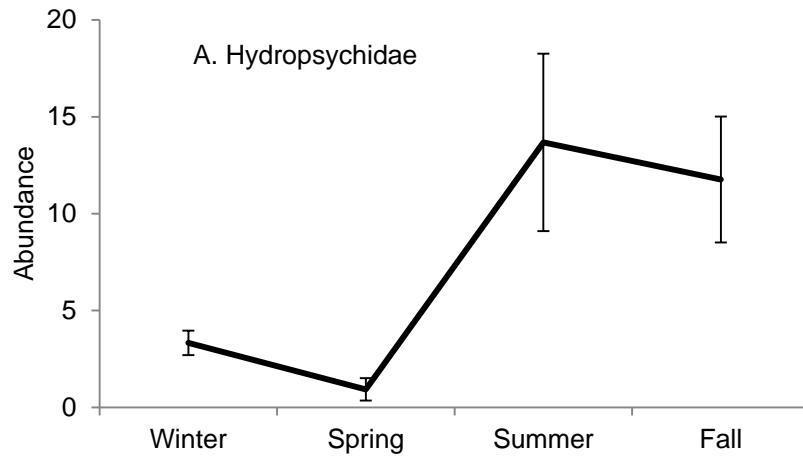


Figure 2.3. Seasonal abundance (mean  $\pm$  s.e.) of selected invertebrate families colonizing leaf litter in headwater streams of hemlock- and deciduous-dominated canopies in central Appalachia. A. Hydropsychidae; B. Chironomidae; C. Leuctridae; D. Peltoperlidae.



## **Chapter 3:**

### **Effects of invertebrate exclusion on litter breakdown in Appalachian streams threatened by hemlock decline**

#### Introduction

Headwater streams in the temperate zone receive annual inputs of allochthonous leaf litter from riparian vegetation which is decomposed and transported downstream (Fisher and Likens 1973). Litter processing in these streams is an important ecosystem process (Lowe and Likens 2005, MacDonald and Coe 2007) in part because decomposing litter produces coarse and fine particulate organic matter (CPOM and FPOM, respectively), and dissolved organic matter (DOM). All are important resources for numerous organisms in situ and downstream (Wallace et al. 1997).

As a resource for consumers, litter varies considerably in quality among plant taxa (Webster and Benfield 1986). Deciduous trees often produce high quality litter that is readily colonized by microbes and invertebrates. Conversely, coniferous or evergreen trees usually produce poor quality, low nutritive litter which has significant physical and chemical barriers retarding utilization by microbes and invertebrates (Webster and Benfield 1986, Davies and Boulton 2009). However, some evergreens produce greater and more continuous annual litter inputs to streams compared to deciduous counterparts (Pozo et al. 1997) so their importance as a resource should not be overlooked.

Complex interactions between leaf characteristics, microbes, animals, and the stream environment regulate litter decomposition rate and thus FPOM and DOM production. In particular, activity by microbes and invertebrate shredders is considered



integral to converting CPOM to FPOM in streams (Hieber and Gessner 2002). Aquatic fungi metabolize tough plant structural components such as cellulose (Bärlocher 1992, Gessner et al. 2007), condition CPOM for shredder feeding (Webster and Benfield 1986), and contribute significantly to litter breakdown (Hieber and Gessner 2002). Shredders colonize leaves and consume both plant and microbial biomass, physically fragment leaves for further breakdown, and excrete FPOM (Wallace and Webster 1996). Microbes are directly affected by differences in leaf properties such as nutrient content or plant defensive compounds, which impacts decomposition (Gessner et al. 2007). The magnitude of the effects to benthic shredders feeding on these leaves is mediated by colonizing microbes. Thus, if there are changes to existing litter resources, it will likely have significant effects on the decomposer community (Leroy and Marks 2006) and litter processing within streams.

In the central and southern Appalachian region of North America, long-term changes to riparian forest communities are predicted to occur near headwater streams. Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), an exotic herbivore of Asian origin, is causing significant mortality of eastern hemlock (*Tsuga canadensis* (L.) Carrière) by feeding on xylem ray parenchyma cells (Young et al. 1995) and depleting starch reserves. Eastern hemlock is very susceptible to adelgid feeding (Montgomery et al. 2009) and native predators are unable to effectively regulate its populations (Wallace and Hain 2000). Currently, eastern hemlock decline is occurring throughout its range (Evans et al. 2011) but its loss in Appalachia may be profound consequences.

Hemlock is an important component of headwater stream riparian forests in Appalachia, and is considered to be a foundation species (Ellison et al. 2005). It is often the only abundant conifer occurring near streams and when present, can stabilize thermal and hydrological regimes of stream water, influencing fish (Ross et al. 2003) and benthic invertebrate communities (Snyder et al. 2002, Adkins 2012).

Hemlock needles are not considered a high quality resource for invertebrates (Maloney and Lamberti 1995) and have low retention in streams due to narrow size. Nevertheless, hemlock is abundant and prevalent near streams and its litterfall phenology suggests it is a reliably available resource (Adkins and Rieske *in review*). Consequently litter produced by hemlock may be an important resource for stream food webs.

As a conifer in the Pinaceae, hemlock litter breakdown is classified as ‘slow’ (Webster and Benfield 1986). Slow-decomposing litter usually reaches peak microbial conditioning later in the season than fast-decomposing litter, thus it becomes suitable substrate for invertebrate colonization later, and has distinct decomposition dynamics compared to fast-decomposing litter (Petersen and Cummins 1974).

The near loss of hemlock-dominated forests is expected if HWA is left unmitigated in the central and southern Appalachians, to be followed by hardwood species replacement (Spaulding and Rieske 2010, Ford et al. 2012). In these riparian areas, sweet birch (*Betula lenta* L.), American beech (*Fagus grandifolia* Ehrhart), and great laurel (*Rhododendron maximum* L.) co-occur with hemlock and will be involved in its replacement (Spaulding and Rieske 2010, Krapfl et al. 2011, Ford et al. 2012, Adkins and Rieske 2013). Sweet birch is an early-successional species while beech is a late-

successional species. Both have leaves that abscise abruptly in the fall and form a large pulse of litter, with the rate of litter breakdown classified as ‘medium’ (Webster and Benfield 1986). Great laurel is an ericaceous, evergreen understory shrub with a litter breakdown rate classified as ‘slow’ (Webster and Benfield 1986).

Adelgid-induced decline of eastern hemlock and long-term replacement by birch, beech, and rhododendron will alter the composition of litter inputs entering streams. This shift in litter composition is likely to have bottom-up effects on the stream communities which utilize these resources. To determine the extent of these consequences in the wake of adelgid-induced shifts in forest composition, I manipulated microbial and invertebrate colonization of eastern hemlock, sweet birch, American beech, and rhododendron leaves using different sized meshes and monitored breakdown in streams with hemlock-dominated and deciduous-dominated canopies. My overall goal was to understand the potential consequences of hemlock woolly adelgid-induced litter resource changes on stream communities, and to understand the respective contributions of invertebrates and microbes to the decomposition of these litter species.

## Materials & Methods

### *Study Site*

Study sites were located at Kentucky Ridge State Forest (Bell Co.), located in the Central Appalachian ecoregion of Kentucky and characterized by mixed mesophytic forests on steep hills and mountains underlain by Pennsylvanian shale, siltstone, sandstone, and coal. Streams there are typically cool with moderate to high gradients and have a cobble or boulder substrate (Woods et al. 2002). Hemlock woolly adelgid was

initially reported in Bell County in 2007 and although it occurs throughout the forest, no hemlock decline has been noted at my study streams.

Six first and second order perennial streams were selected based on similarities in watershed characteristics (Adkins and Rieske 2013). Vegetation surveys using USDA Forest Service common stand exam protocols were used to characterize riparian vegetation in 2008 (USDA 2009, Adkins and Rieske 2013). Overstory hemlock stem density was used to designate three streams as ‘hemlock-dominated’ and three streams as ‘deciduous-dominated’ (Adkins and Rieske 2013). At each stream, three sampling points were established within a 30 m reach to evaluate litter colonization and breakdown.

### *Litter Bags*

In October 2012, newly abscised leaves of sweet birch and American beech were collected from Kentucky Ridge State Forest; hemlock twigs were clipped from trees and newly fallen rhododendron leaves were collected at Natural Bridge State Resort Park. Leaves were returned to the laboratory and air dried.

Mesh bags were constructed for two treatments: ‘exclusion’ (designed to exclude invertebrates but allow microbial colonization) and ‘open’ (allowing both invertebrate and microbial colonization). ‘Exclusion’ bags ( $17\text{ cm} \times 16\text{ cm} \times 300\text{ }\mu\text{m}^2$ ) were constructed of nylon mesh fabric sewn together using medium weight polyester thread. ‘Open’ bags ( $18\text{ cm} \times 18\text{ cm} \times 10\text{ mm}^2$ ) were constructed of garden fence material sewn together with 0.7 mm diameter nylon fishing line, and with a window mesh sleeve inserted ( $16\text{ cm} \times 16\text{ cm} \times 1\text{ mm}^2$ ) to help retain leaf material. In each bag 5.5 g litter of one species was sealed.

At each sampling point, four 0.6 m lengths of 12 mm rebar were anchored in the streambed, and one mesh bag per treatment of each litter species was randomly attached at 0.25 m intervals along the length of a steel cable (2.42 m × 3.2 mm), so that attached to each cable were eight mesh bags. One cable with mesh bags was attached to each embedded rebar and weighted down to ensure that all bags were at least partially submerged. There were a total of 288 mesh bags, 144 of each mesh treatment (exclusion versus open), 72 of each litter species, 144 in streams with hemlock-dominated riparian canopy, and 144 in streams with deciduous-dominated riparian canopy. Bags were deployed on 19 November 2012 to allow for invertebrate colonization.

#### *Litter Colonization*

One cable, consisting of four open and four exclusion treatment bags containing each a single litter species, was removed from each sample point at 3 and 5 months (March 1 and May 3). Mesh bags were individually sealed in 3.7 L plastic bags containing ~100 ml 70% ethanol, and returned to the laboratory, where invertebrates were separated from leaf material, counted, identified to family, and assigned to functional feeding groups, including collector – filterers, collector – gatherers, predators, scrapers, and shredders according to Merritt et al. (2008), and stored in 70% ethanol.

#### *Litter Breakdown*

After deployment, subsamples of each litter species were retained, air-dried for 3 d, dried at 60 °C for 4 d, and weighed to calculate the approximate oven-dried mass contained within each litter bag. Leaves recovered from the mesh bags were washed with tap water and air-dried for 3-6 d. A sub-sample of ~10% air-dried mass was removed after 1 d from each sample to analyze microbial colonization (see Appendix 3.1). The

remaining leaf sample was oven-dried at 60 °C for 4 d, and weighed to the nearest 0.1 g to calculate dry mass remaining. Sub-samples of oven-dried litter were combusted at 500 °C for 5 hours to calculate the ratio of organic to inorganic matter and convert dry mass remaining to ash-free dry mass remaining. Mass remaining was expressed as a percentage of the original mass and the natural logarithm of sample % mass remaining was regressed against time (days) to estimate the rate of decay ( $k$ ) for each litter species at each transect using the exponential decay model  $M_t = M_0 e^{-k t}$ , where  $M_t$  is the mass remaining at time  $t$  (days), and  $M_0$  is the mass remaining at time zero (Bärlocher 2005).

### *Analysis*

Invertebrate colonization (number of invertebrates) was compared across riparian canopy (hemlock and deciduous), litter species (birch, beech, rhododendron, and hemlock), treatment (open and exclusion), and season (winter and spring). These factors were the main effects used in a four-way repeated measures analysis of variance (ANOVA, PROC GLM, SAS v.9.1.3) with a split-plot factorial design. Season was the repeated measure, riparian canopy was the whole plot and litter species was the split plot and the factorials were the open and exclusion treatments. Decay rate,  $k$ , for leaves in mesh bags was  $\sqrt{n} + 0.5$  transformed to meet normality assumptions and analyzed using a three-way ANOVA, with riparian canopy, litter species, and mesh treatment as main effects. The difference of least squares was used to separate means for litter colonization and litter breakdown.

## Results

### *Litter Colonization*

Litter colonization in open bags was greater than in exclusion bags ( $F_{1,28} = 36.59$ ,  $P < 0.0001$ ). Of the 11,367 invertebrates colonizing litter, 68% (7,758) were collected in open treatment bags and 32% (3,609) in exclusion treatment bags. Invertebrate colonization was influenced by season ( $F_{1,28} = 93.93$ ,  $P < 0.0001$ ), and a weakly significant litter species effect ( $F_{3,28} = 2.78$ ,  $P < 0.06$ ). The only difference among litter species was greater colonization of hemlock compared to rhododendron. Season influenced colonization ( $F_{1,28} = 93.93$ ,  $P < 0.0001$ ), and there was a significant mesh treatment  $\times$  season interaction ( $F_{1,28} = 7.54$ ,  $P < 0.05$ ). Seasonally, invertebrate colonization was greater in spring than winter and the difference between open and exclusion bags increased in spring (Fig. 3.1). Riparian canopy had no effect on litter colonization.

Invertebrates belonged to 35 families in nine orders (Appendix 3.2). Dominant families included Chironomidae (37%) and Leuctridae (32%). The colonizer community was dominated by shredders (50%) and collector-gatherers (43%), while predators (5%), collector-filterers (2%), and scrapers (<1%) were also present.

Chironomidae were only weakly affected by mesh treatment ( $F_{1,28} = 3.07$ ,  $P = 0.09$ ) but colonization by Leuctridae was reduced in exclusion bags ( $F_{1,28} = 15.17$ ,  $P < 0.001$ ) (Table 3.1). Both shredder and collector-gatherer colonization was greatly reduced in exclusion bags ( $F_{1,28} = 46.36$ ,  $P < 0.0001$  and  $F_{1,28} = 13.84$ ,  $P < 0.001$ ,

respectively), and increased seasonally over the course of the experiment (shredders:  $F_{1,28} = 140.95$ ,  $P < 0.0001$ ; collector-gatherers:  $F_{1,28} = 19.65$ ,  $P < 0.001$ ) (Table 3.1). Litter species had a weakly significant influence on shredders ( $F_{3,28} = 2.84$ ,  $P < 0.06$ ), and there was a weakly significant litter species  $\times$  mesh treatment interaction ( $F_{3,28} = 2.63$ ,  $P = 0.07$ ). Shredder colonization was only reduced in exclusion bags containing beech or hemlock litter. For birch and rhododendron, the difference between mesh treatments was not significant (Fig. 3.2).

### *Litter Breakdown*

Litter breakdown rate ( $k$ ) was influenced by mesh treatment ( $F_{1,114} = 71.14$ ,  $P < 0.0001$ ) and litter species ( $F_{3,114} = 54.45$ ,  $P < 0.0001$ ), and a significant mesh treatment  $\times$  litter species interaction ( $F_{3,114} = 3.00$ ,  $P < 0.05$ ). Litter breakdown rate was more rapid in open bags compared to exclusion bags (Fig. 3.3), regardless of litter species, but when considering individual litter species across both mesh sizes, breakdown rates differed. Beech and rhododendron decomposition was slowest ( $k = -0.00273 \pm 0.0003$  and  $-0.00388 \pm 0.0007$ , respectively) but hemlock and birch broke down rapidly ( $k = -0.00795 \pm 0.0005$  and  $-0.00832 \pm 0.001$ , respectively) (Fig. 3.4). In open bags breakdown rate was accelerated for all litter species and percent mass remaining was generally lower in open bags; however hemlock percent mass remaining showed the smallest numerical differences, especially at 102 d (Fig. 3.5).



## Discussion

### *Litter Colonization*

My mesh treatments were not completely effective in manipulating invertebrate invertebrate colonization of my sample litter, but colonization in exclusion bags was ~50% less than in open bags, except for colonization by shredders and Chironomidae, which were unaffected by mesh size. Shredders colonized birch and rhododendron litter equally across mesh treatments and chironomid midges were equally abundant in all litter across mesh treatments. The cause of this lack of difference in shredder colonization is unclear but the dominant shredders (leuctrid stoneflies) were significantly inhibited by exclusion mesh. Chironomid larvae are small, allowing them easy access through both meshes. Despite these deviations, only small differences in the functional feeding group composition of invertebrates colonizing litter in open and exclusion bags existed. This indicates that mesh treatment only reduced the magnitude of colonization by functional groups and did not favor or hinder colonization by specific groups. Moreover, when invertebrate colonization increased from winter to spring, mesh treatment differences actually increased, showing that the exclusion mesh continued to inhibit invertebrates despite changing invertebrate abundance and significant time spent in the streams.

### *Litter Breakdown*

Mass loss of litter in the open treatment is essentially due to endemic invertebrate and microbe activities and their interactions. In contrast, in the exclusion treatment invertebrate abundance, and therefore invertebrate activity, was reduced, and the litter breakdown I document is more attributable to microbial breakdown, as there was a ~50% reduction in invertebrates. Over the course of this study, 25 to 75 percent of litter mass

was lost from processing by the environment, invertebrates, and microbes. Mass loss was accelerated in the open treatment where greater amounts of invertebrates could access and break down litter and in part by wider mesh openings which may have allowed larger leaf fragments to exit bags.

Shredders are most often implicated in increasing rates of litter breakdown (Graca 2001), but collector-gatherers are also important and can work in tandem with shredders to process litter (Dieterich et al. 1997). Shredder and collector-gatherer abundance was reduced by approximately 66% and 50%, respectively, in exclusion bags. Their contribution to breakdown is evident in accelerated decay rate ( $k$ ) in open bags and in the percent mass loss increases in open bags relative to exclusion bags, which typically exceeded 15% after 165 d, except in hemlock litter (Fig. 3.5). Despite more rapid decay rates for hemlock litter exposed to more invertebrates, percent hemlock mass remaining was essentially equal between treatments at 102 d and only increased to 12% at 165 d. This suggests that invertebrates may not be crucial to early hemlock litter processing in these streams, which, given hemlock's generally accepted poor nutritional quality (Webster and Benfield 1986, Maloney and Lamberti 1995) and its low retention in streams, is not unexpected. Instead, microbes may have greater importance in hemlock's breakdown.

Other studies have noted microbial dominance in breaking down tough, poor quality litter (Gessner and Chauvet 1994). Hemlock needles are expected to have more structural components and higher levels of defensive chemicals than birch, beech, or rhododendron litter (Webster and Benfield 1986). Given microbes superior enzymatic ability to invertebrates in breaking down tough plant material and inhibitory chemicals

(Bärlocher 1992, Gessner et al. 2007), it follows that microbes could be the dominant drivers of hemlock decomposition here. This difference has implications for Appalachian streams where hemlock litter inputs, which appear to rely on microbial breakdown, will decline while increasing inputs from birch, beech, and rhododendron involve both invertebrate and microbial processing.

### *Conclusions*

Broadleaved litter represents the resources available to Appalachian headwater streams after hemlock woolly adelgid-induced hemlock decline and replacement. My results suggest that loss of hemlock litter may not have a strong impact on invertebrates in these streams, but increasing inputs from birch, beech, and rhododendron may. Hemlock litter is a low quality resource compared to broadleaved litter due to physical and chemical barriers, low retention in streams and the relatively fast breakdown reported here.

Changes in litter inputs have consequences for both aquatic and terrestrial food webs. The majority of invertebrates collected in this study are insects with winged adult forms. While immature aquatic insects form an important linkage in the stream food web, emergent adults leave the water and can be an important part of the terrestrial food web (Henschel et al. 2001). Thus, higher trophic levels could be affected by the increased importance of the benthic invertebrate community in processing litter inputs entering these streams.

Clearly, further investigation into the role of the microbial community in breakdown of hemlock and other litter species is warranted. Despite its poor qualities,

hemlock litter is currently a major source of energy to these streams and its processing may rely heavily on microbial decomposers. The implications of the loss of hemlock litter after riparian hemlock decline may not be significant for invertebrates, but microbes could be markedly and more directly affected by this change.

Tables and Figures

Table 3.1. Abundance (mean  $\pm$  s.e.) of all invertebrates, shredders, collector-gatherers, Chironomidae, and Leuctridae colonizing leaf litter exposed in open and exclusion bags in headwater streams of central Appalachia from winter to spring. Means with the same letter within each effect are not significantly different ( $P < 0.05$ ).

Effect	Treatment	Total	Shredders	Collector-Gatherers	Chironomidae	Leuctridae
Mesh	Open	53.88 (4.34) b	29.56 (3.25) b	20.37 (1.60) b	17.78 (1.57) a	28.82 (3.04) b
	Exclusion	25.06 (2.34) a	10.22 (1.48) a	13.63 (1.34) a	15.07 (1.33) a	13.21 (1.68) a
Litter species	Birch	37.83 (4.67) ab	18.54 (2.93) ab	16.43 (2.39) ab	15.8 (2.32) a	16.57 (2.61) a
	Beech	43.07 (6.33) ab	23.21 (4.95) b	17.07 (1.96) ab	16.85 (1.85) a	24.45 (4.79) a
	Rhododendron	28.64 (3.73) a	13.49 (2.60) a	13.39 (1.56) a	12.31 (1.38) a	14.15 (2.32) a
	Hemlock	48.33 (5.50) bc	24.32 (3.97) b	21.11 (2.40) bc	20.7 (2.42) a	26.2 (4.07) a
Season	Winter	22.01 (2.17) a	6.04 (0.89) a	13.81 (1.43) a	13.75 (1.43) a	4.77 (0.48) a
	Spring	56.92 (4.22) b	33.74 (3.22) b	20.19 (1.52) b	18.86 (1.46) b	28.63 (2.90) b

Figure 3.1. Invertebrate colonization ((mean  $\pm$  s.e.)) of leaf litter exposed in open (dashed line) and exclusion (solid line) bags in headwater streams in central Appalachia from fall to spring.

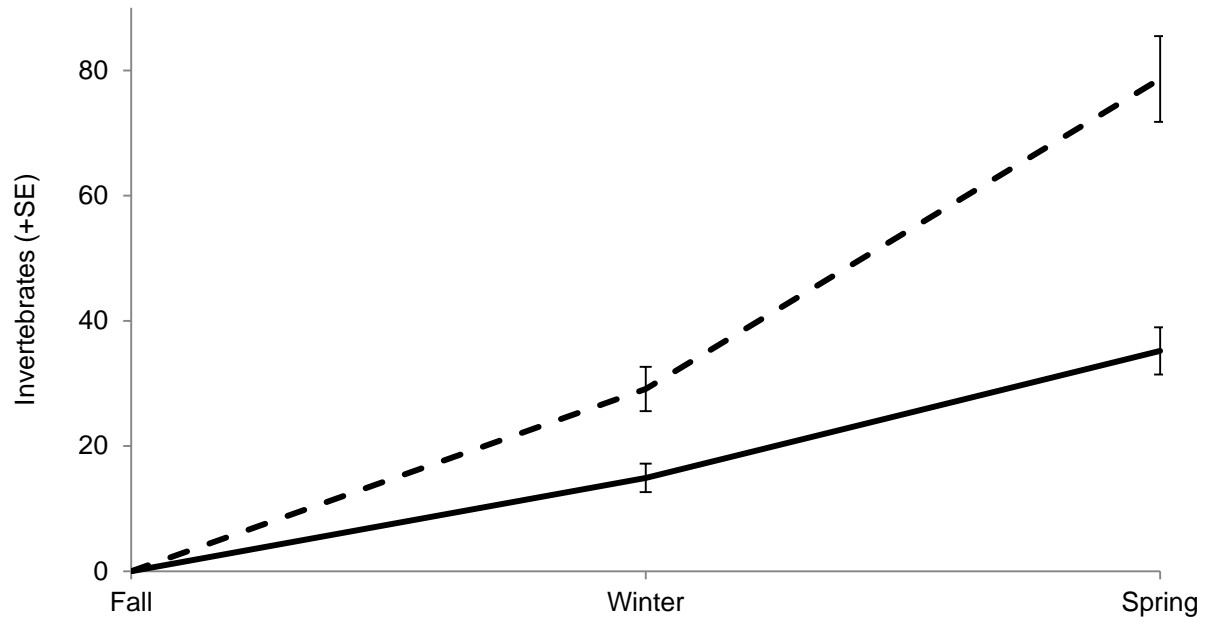


Figure 3.2. Benthic shredder colonization of four species of litter in open (□) and exclusion (▨) bags in headwater streams in central Appalachia. \* indicates statistical significance ( $P < 0.05$ ).

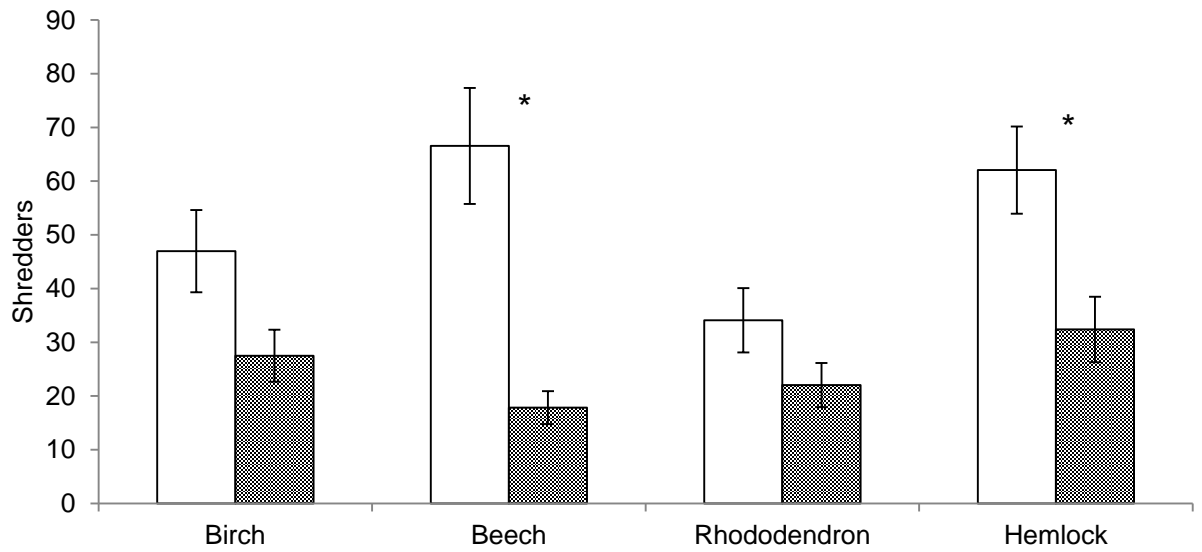


Figure 3.3. Litter breakdown of four tree species expressed as % mass remaining in open (dashed lines) and exclusion (solid lines) bags placed in headwater streams in central Appalachia in fall (0 d), winter (102 d), and spring (165 d).

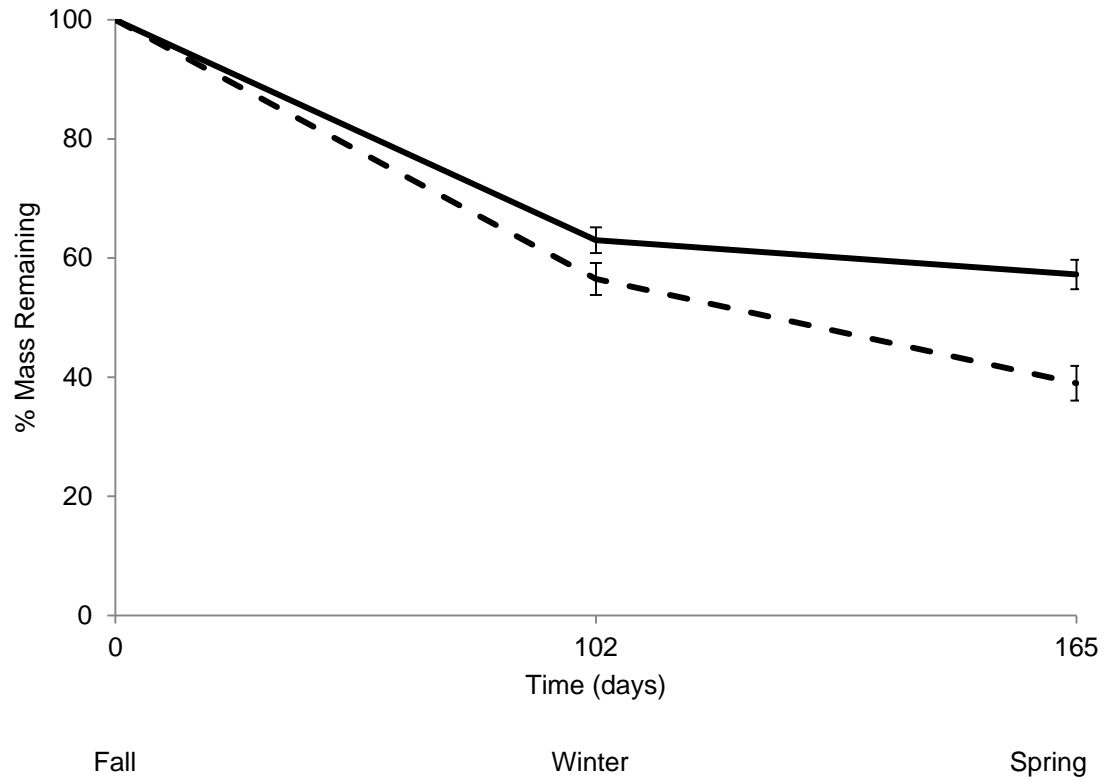




Figure 3.4. Percent of birch, beech, rhododendron, and hemlock leaf mass remaining in mesh bags in headwater streams in central Appalachia in fall (0 d), winter (102 d), and spring (165 d).

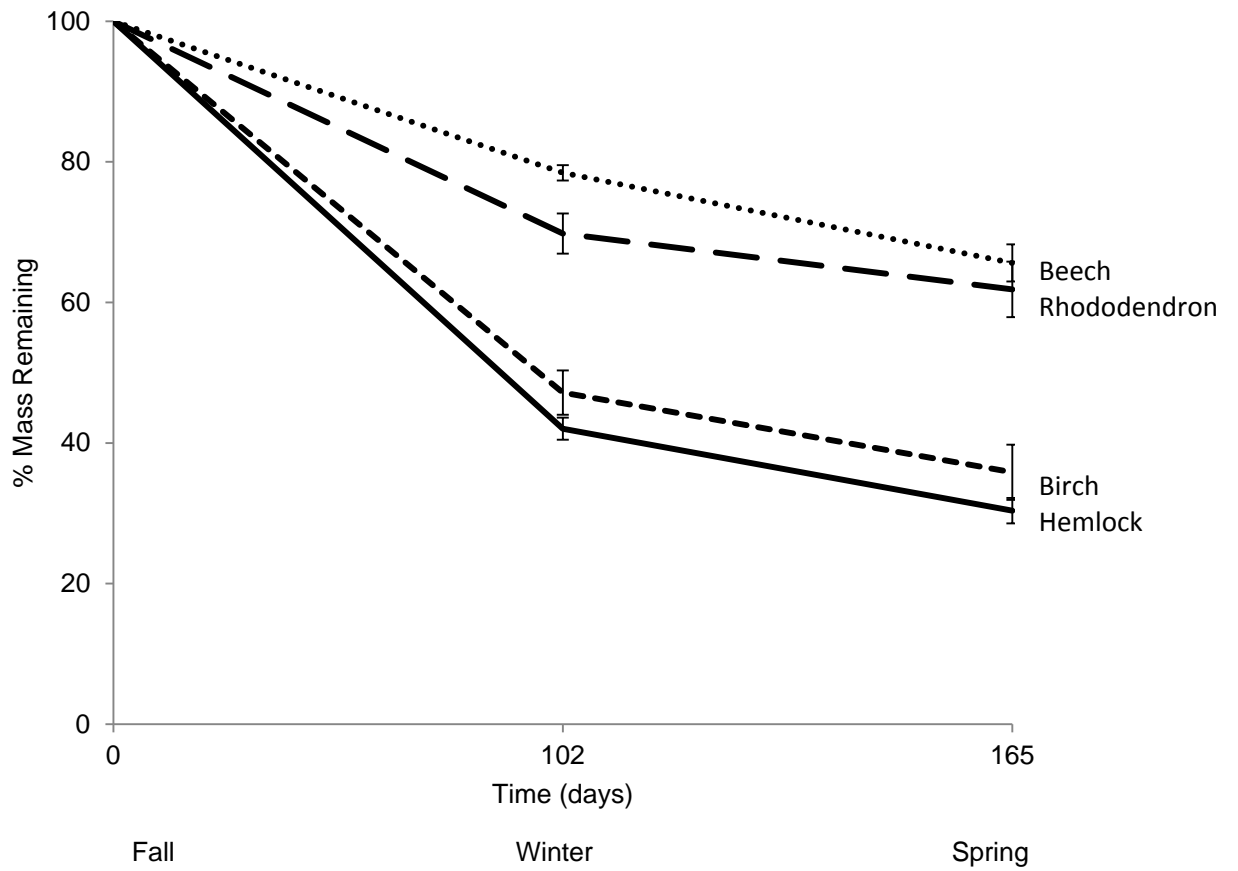
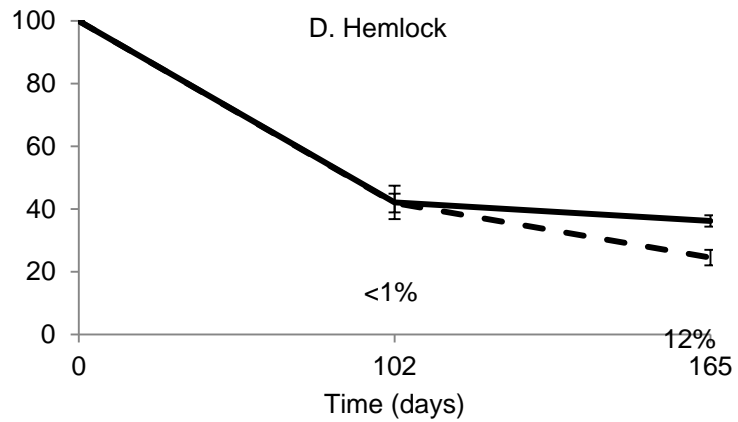
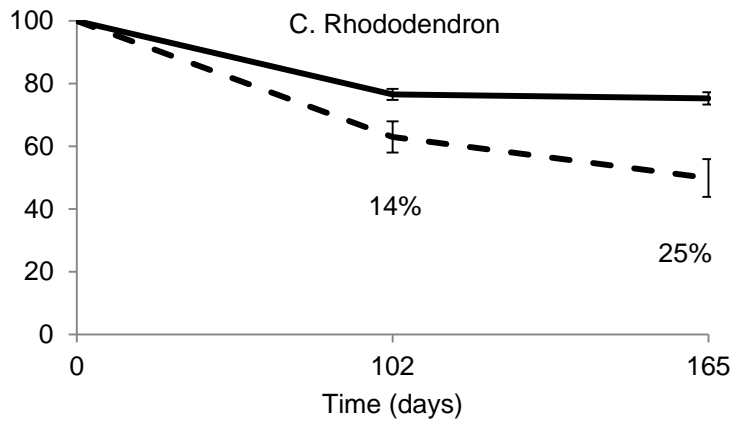
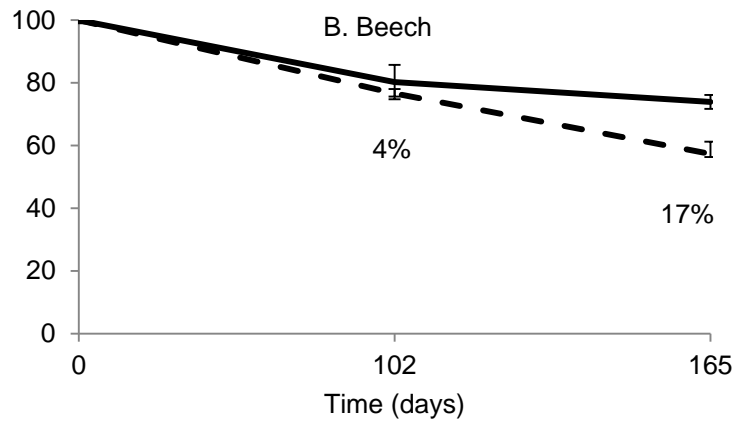
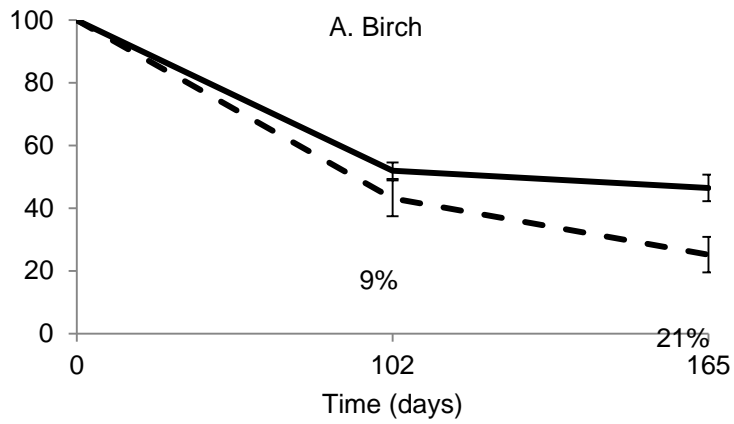


Figure 3.5. Percent of litter mass remaining in exclusion (solid lines) and open (dashed lines) treatment bags placed in headwater streams in central Appalachia in fall (0 d), winter (102 d), and spring (165 d). Percent mass difference between mesh treatments shown. A. Birch; B. Beech; C. Rhododendron; D. Hemlock.

55



## Appendices

Appendix 2.1. Characteristics of headwater streams with hemlock-dominated and deciduous-dominated canopies in southeastern Kentucky (Kentucky Ridge State Forest).

### *Methods*

The pH, dissolved oxygen, and conductivity of stream water were measured using a YSI 556 Multi-Probe System (YSI Inc., Marion, MA) immediately downstream of the first sampling point. Two 250 ml water samples were collected concurrently in 710 ml plastic bags, sealed, returned to the laboratory and stored at 4 °C for chemical analysis.

Stored samples were evaluated for carbon using a TOC-V<sub>CSN</sub> Analyzer (Shimadzu Inc., Columbia, MD). Total carbon was analyzed without preparation; samples were acidified to remove inorganic carbon for total organic carbon analysis; samples were acidified and filtered through 45 µm filter paper to analyze dissolved organic carbon.

Stored samples were evaluated for nitrogen using an Autoanalyzer III (Bran & Luebbe, Delavan, WI). Samples were reduced via sulfate / copper-sulfate to analyze nitrate; samples underwent a phenol nitrogen reaction to analyze ammonia. Samples were contained in ampoules, autoclaved, and mixed with sodium persulfate to analyze total nitrogen; after filtering through 45 µm filter paper, the same process was used to analyze dissolved nitrogen.

### *Results*

Riparian canopy affected stream pH ( $F_{1,16} = 10.02$ ,  $P < 0.01$ ), and was greater in hemlock-dominated streams than deciduous-dominated streams (Table A), but there

were no seasonal differences. Dissolved oxygen and conductivity in streams did not differ across riparian canopies or seasons.

No differences were detected in nitrate, ammonium, total nitrogen, dissolved nitrogen, total carbon, total organic carbon, or total dissolved carbon concentrations based on riparian canopy, but seasonal differences in several parameters were evident. Season influenced concentrations of ammonium ( $F_{3,16} = 13.94$ ,  $P < 0.01$ ), total nitrogen ( $F_{3,16} = 5.04$ ,  $P < 0.05$ ), dissolved nitrogen ( $F_{3,16} = 7.62$ ,  $P < 0.01$ ), and total organic carbon ( $F_{3,16} = 4.48$ ,  $P < 0.05$ ) (Table A).

### *Discussion*

Streams with deciduous-dominated canopies had lower pH than hemlock-dominated streams. Riparian vegetation can influence stream chemistry in a variety of ways (Dosskey et al. 2010). Other studies, including those conducted in the same streams that I evaluated, found hemlock-dominated streams to have lower pH than their deciduous counterparts (Adkins 2012), or to have no difference in pH (Snyder et al. 2002). It is unclear why pH measurements in my study were lower in deciduous-dominated streams.

Fluctuations in ammonium, dissolved nitrogen, and total nitrogen occurred seasonally. These seasonal changes, although significant, were relatively small numerically and unlikely to be biologically relevant. Headwater streams are important facilitators of nitrogen export from the atmosphere to larger bodies of water (Peterson et al. 2001), and my observed changes may be a reflection of small differences in the rate of nitrogen export in these streams.

Total organic carbon increased more than two fold during the period of this study. Levels of total organic carbon are indicative of decaying organic matter in water and are related to stream flow (Fisher and Likens 1973). I did not measure stream flow, but it did change seasonally (personal observation) and may explain why total organic carbon levels changed.

Table A. Headwater stream characteristics (mean ( $\pm$  s.e.)) in hemlock- and deciduous-dominated canopies of central Appalachia over one year. Means with the same letter within each effect are not significantly different ( $P < 0.05$ ).

Effect	Canopy		Season			
	Hemlock	Deciduous	Winter	Spring	Summer	Fall
pH	6.31 (0.11) b	5.49 (0.21) a	5.72 (0.33) a	6.11 (0.38) a	5.85 (0.28) a	5.90 (0.15) a
Dissolved oxygen <sup>1</sup>	9.93 (0.33) a	9.81 (0.28) a	10.46 (0.12) a	9.36 (0.45) a	10.33 (0.57) a	9.32 (0.26) a
Conductivity ( $\mu\text{S cm}^{-1}$ )	36.01 (9.64) a	47.23 (10.44) a	37.98 (11.45) a	44.5 (15.03) a	50.33 (16.64) a	33.67 (15.73) a
Nitrate <sup>1</sup>	0.05 (0.02) a	0.08 (0.03) a	0.14 (0.07) a	0.03 (0.02) a	0.01 (0.01) a	0.07 (0.03) a
Ammonium <sup>1</sup>	0.05 (0.01) a	0.07 (0.02) a	0.0 (0.0) a	0.08 (0.02) b	0.05 (0.01) b	0.12 (0.02) c
Total nitrogen <sup>1</sup>	2.57 (0.07) a	2.50 (0.06) a	2.52 (0.09) b	2.68 (0.06) b	2.31 (0.06) a	2.65 (0.09) b
Dissolved nitrogen <sup>1</sup>	2.49 (0.06) a	2.53 (0.06) a	2.73 (0.06) c	2.52 (0.05) b	2.35 (0.07) a	2.47 (0.06) b
Total carbon <sup>1</sup>	13.53 (2.03) a	19.27 (3.59) a	9.49 (1.92) a	15.76 (3.04) a	17.63 (4.06) a	22.73 (5.84) a
Total organic carbon <sup>1</sup>	11.90 (2.00) a	16.61 (3.15) a	6.60 (1.22) a	15.85 (2.89) b	14.44 (3.56) b	20.14 (4.92) b
Dissolved organic carbon <sup>1</sup>	9.69 (1.91) a	12.91 (2.65) a	6.34 (1.17) a	10.06 (1.58) a	13.10 (3.52) a	15.72 (4.79) a

<sup>1</sup> mg

Appendix 2.2. Total abundance of invertebrate families colonizing leaf litter in headwater streams with hemlock-dominated and deciduous-dominated canopies in central Appalachia over one year.

Order	Family	Total	Canopy			Litter			Season			
			Deciduous	Hemlock	Birch	Beech	Rhododendron	Hemlock	Winter	Spring	Summer	Fall
Coleoptera	Elmidae	24	14	10	2	8	9	5	3	3	13	5
	Psephenidae	11	6	5	2	1	3	5	1	2	3	5
	Carabidae	6	5	1	2	1	2	1	0	6	0	0
	Curculionidae	5	3	2	0	0	4	1	2	2	0	1
	Staphylinidae	4	2	2	1	1	1	1	0	4	0	0
Collembola	Tomoceridae	495	325	170	110	155	140	90	16	230	113	136
	Isotomidae	306	43	263	34	247	16	9	0	264	13	29

## Appendix 2.2 (continued)

	Entomobryidae	11	1	10	0	0	10	1	0	10	1	0
	Sminthuridae	4	3	1	1	1	2	0	0	0	2	2
Decapoda	Cambaridae	3	0	3	1	0	2	0	0	1	2	0
Diptera	Chironomidae	1809	671	1138	246	608	538	417	291	708	439	371
	Tipulidae	254	170	84	44	106	61	43	28	56	46	124
	Ceratopogonidae	118	58	60	13	63	38	4	2	12	10	94
	Dixidae	103	52	51	24	25	39	15	7	42	40	14
	Simuliidae	32	27	5	11	11	3	7	15	2	15	0
Ephemeroptera	Ephemerellidae	468	41	427	135	85	128	120	103	3	144	218
	Leptophlebiidae	72	17	55	4	43	18	7	15	28	11	18
	Baetidae	61	27	34	19	4	25	13	50	6	4	1
	Heptageniidae	12	6	6	2	2	3	5	5	1	2	4
	Ameletidae	2	1	1	1	0	1	0	2	0	0	0
Isopoda	Asellidae	152	131	21	47	33	47	25	121	25	0	6



## Appendix 2.2 (continued)

Neuroptera	Corydalidae	7	1	6	1	4	1	1	0	2	2	3
Odonata	Gomphidae	37	18	19	5	24	6	2	7	3	6	21
	Cordulegastridae	12	6	6	0	8	2	2	0	3	0	9
	Aeshnidae	1	0	1	0	0	0	1	0	0	0	1
	Coenagrionidae	1	0	1	0	0	1	0	0	0	0	1
Plecoptera	Leuctridae	1480	924	556	419	625	264	172	193	441	271	575
	Peltoperlidae	728	355	373	158	221	238	111	214	261	177	76
	Nemouridae	448	306	142	107	146	82	113	414	24	10	0
	Perlodidae	364	215	149	82	90	112	80	80	17	180	87
	Chloroperlidae	67	54	13	21	27	13	6	30	20	16	1
	Perlidae	4	3	1	0	1	3	0	1	0	1	2
Trichoptera	Hydropsychidae	2003	833	1170	529	299	644	531	230	67	930	776
	Lepidostomatidae	477	192	285	96	122	143	116	220	201	54	2
	Limnephilidae	22	6	16	5	5	11	1	3	5	4	10

Appendix 2.2 (continued)

Rhyacophilidae	22	11	11	3	5	7	7	9	0	3	10
Philopotamidae	7	5	2	0	0	6	1	1	0	1	5
Molannidae	6	1	5	1	3	2	0	0	0	2	4
Uenoidae	6	3	3	1	3	2	0	3	0	0	3
Hydroptilidae	3	0	3	1	0	2	0	0	2	1	0
Polycentropodidae	2	2	0	0	0	1	1	0	1	0	1
Glossosomatidae	1	1	0	0	0	0	1	0	1	0	0
Psychomiidae	1	0	1	0	1	0	0	0	1	0	0

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Appendix 2.3. Total abundance of invertebrate families collected in kick-net samples from headwater streams with hemlock-dominated and deciduous-dominated canopies in central Appalachia over one year.

Order	Family	Total	Canopy		Season			
			Deciduous	Hemlock	Winter	Spring	Summer	Fall
Coleoptera	Elmidae	111	13	98	42	22	2	45
	Psephenidae	29	7	22	1	1	11	16
	Curculionidae	3	2	1	2	0	1	0
	Dytiscidae	1	0	1	0	1	0	0
	Staphylinidae	1	0	1	1	0	0	0
Collembola	Tomoceridae	68	44	24	3	16	5	44
	Isotomidae	44	19	25	0	40	2	2
	Entomobryidae	12	9	3	11	1	0	0
	Sminthuridae	4	1	3	1	1	0	2
Decapoda	Cambaridae	9	3	6	1	3	3	2
Diptera	Chironomidae	451	166	285	93	115	94	149

## Appendix 2.3 (continued)

	Tipulidae	143	92	51	26	34	40	43
	Ceratopogonidae	39	21	18	12	4	4	19
	Simuliidae	19	15	4	1	4	4	10
	Dixidae	7	3	4	0	3	0	4
Ephemeroptera	Heptageniidae	220	38	182	40	119	29	32
	Baetidae	145	38	107	43	66	16	20
	Leptophlebiidae	63	11	52	31	13	8	11
	Ameletidae	15	5	10	15	0	0	0
	Ephemerellidae	7	2	5	1	1	4	1
	Siphonuridae	1	0	1	1	0	0	0
Isopoda	Asellidae	88	50	38	28	27	0	33
Neuroptera	Corydalidae	2	0	2	0	1	0	1
Odonata	Gomphidae	32	5	27	3	8	7	14
	Cordulegastridae	13	5	8	2	3	3	5

## Appendix 2.3 (continued)

Plecoptera	Leuctridae	373	135	238	85	169	57	62
	Peltoperlidae	114	21	93	79	13	11	11
	Perlodidae	65	21	44	11	30	9	15
	Chloroperlidae	64	30	34	8	19	13	24
	Nemouridae	52	28	24	51	1	0	0
	Perlidae	45	15	30	5	1	21	18
Trichoptera	Hydropsychidae	445	176	269	40	40	66	299
	Philopotamidae	45	5	40	24	1	10	10
	Polycentropodidae	44	12	32	0	5	9	30
	Lepidostomatidae	23	5	18	18	4	1	0
	Rhyacophilidae	13	4	9	2	5	5	1
	Uenoidae	9	4	5	9	0	0	0
	Glossosomatidae	7	2	5	1	1	4	1
	Hydroptilidae	3	1	2	1	0	0	2

Appendix 2.3 (continued)

Limnephilidae	2	1	1	0	2	0	0
Psychomiidae	2	0	2	1	1	0	0
Brachycentridae	1	0	1	0	0	1	0
Goeridae	1	1	0	0	0	1	0

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Appendix 2.4. Total abundance of invertebrate families collected in leaf-pack samples from headwater streams with hemlock-dominated and deciduous-dominated canopies in central Appalachia over one year.

Order	Family	Total	Canopy		Season			
			Deciduous	Hemlock	Winter	Spring	Summer	Fall
Coleoptera	Elmidae	32	16	16	2	0	19	11
	Staphylinidae	6	6	0	5	1	0	0
	Curculionidae	3	2	1	2	0	0	1
	Carabidae	2	0	2	0	2	0	0
	Psephenidae	1	0	1	0	0	0	1
Collembola	Tomoceridae	237	188	49	0	25	27	185
	Isotomidae	58	44	14	3	4	2	49
	Entomobryidae	39	32	7	39	0	0	0
	Sminthuridae	9	8	1	0	0	2	7
Decapoda	Cambaridae	3	3	0	0	0	1	2
Diptera	Chironomidae	391	183	208	125	48	74	144

## Appendix 2.4 (continued)

	Ceratopogonidae	107	105	2	0	1	104	2
	Tipulidae	102	79	23	17	17	9	59
	Dixidae	96	49	47	6	18	36	36
	Simuliidae	9	1	8	4	0	4	1
Ephemeroptera	Heptageniidae	45	26	19	4	1	14	26
	Ephemerellidae	44	12	32	9	1	10	24
	Leptophlebiidae	13	1	12	7	6	0	0
	Baetidae	3	1	2	1	1	0	1
Isopoda	Asellidae	64	56	8	34	5	2	23
Neuroptera	Corydalidae	1	1	0	0	0	0	1
Odonata	Gomphidae	25	22	3	7	1	12	5
	Cordulegastridae	1	0	1	0	0	0	1
Plecoptera	Peltoperlidae	698	378	320	205	102	258	133
	Leuctridae	400	257	143	131	51	70	148



Appendix 2.4 (continued)

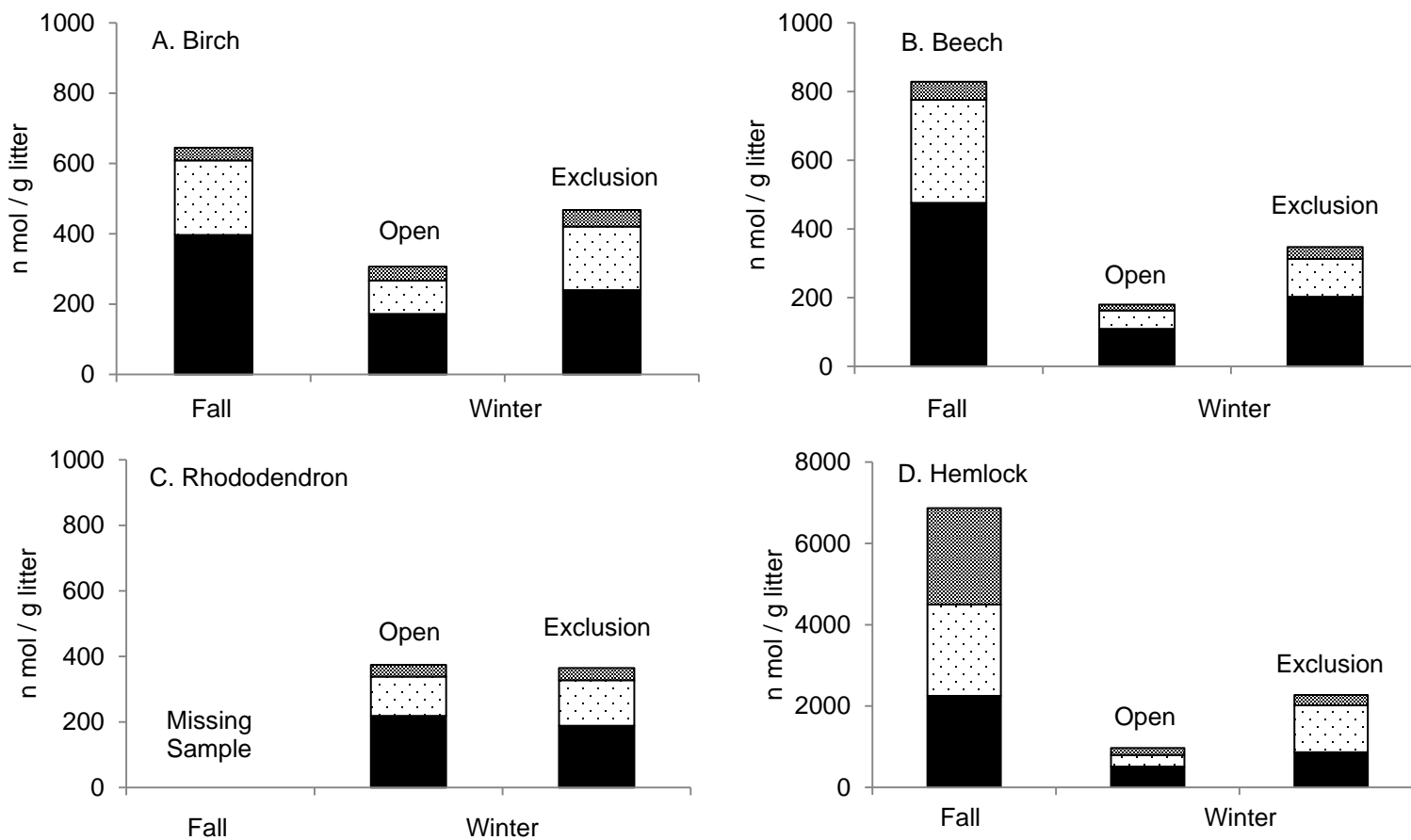
	Perlodidae	79	45	34	15	6	18	40
	Nemouridae	78	52	26	67	8	2	1
	Chloroperlidae	14	9	5	14	0	0	0
	Perlidae	6	3	3	0	0	2	4
Trichoptera	Hydropsychidae	101	74	27	52	0	18	31
	Lepidostomatidae	69	21	48	28	28	7	6
	Rhyacophilidae	14	10	4	2	0	3	9
	Limnephilidae	6	2	4	3	2	1	0
	Philopotamidae	4	1	3	2	0	2	0

### Appendix 3.1. Analysis of microbial colonization.

After air-drying for 1d, litter samples were weighed and ~10% of each litter sample was removed and kept inside paper bags for freeze-drying. Samples were freeze-dried for 4 d, sealed inside a Ziploc bag and stored at room temperature for eventual grinding and lipid extraction in order to perform phospholipid fatty acid analysis (PLFA). For the preliminary analysis, a single sample of birch, beech, rhododendron, and hemlock source litter and litter collected from both mesh treatments after 102 d was used. Extracted lipids were identified and measured using gas chromatography and labeled as being associated with plants, fungi, or gram +/- bacteria based on known lipid markers (for an example see Gilliam et al. 2010).

Figure A. Preliminary results of PLFA on source leaf litter (fall) and on leaf litter exposed in open and exclusion mesh in headwater streams for 102 d (winter). Bars show lipid quantity (n mol) per g litter associated with plants (■), fungi (▨), and bacteria (▩).

72



Appendix 3.2. Total abundance of invertebrate families colonizing leaf litter exposed in open and exclusion mesh in headwater streams with hemlock-dominated and deciduous-dominated canopies in central Appalachia.

Order	Family	Mesh Treatment			Litter Species				Canopy		Season	
		Total	Open	Exclusion	Birch	Beech	Rhododendron	Hemlock	Deciduous	Hemlock	Winter	Spring
Coleoptera	Staphylinidae	43	34	9	10	6	17	10	32	11	31	12
	Carabidae	7	6	1	4	3	0	0	6	1	1	6
	Elmidae	7	6	1	3	1	1	2	2	5	3	4
	Curculionidae	2	2	0	2	0	0	0	2	0	0	2
	Psephenidae	1	1	0	0	1	0	0	1	0	0	1
Collembola	Isotomidae	422	344	78	88	128	121	85	171	251	272	150
	Tomoceridae	104	100	4	29	15	34	26	67	37	22	82
	Sminthuridae	3	3	0	1	1	0	1	1	2	1	2

## Appendix 3.2 (continued)

Decapoda	Cambaridae	5	5	0	1	1	2	1	2	3	2	3
Diptera	Chironomidae	4201	2347	1854	1027	1045	763	1366	1818	2383	1636	2565
	Ceratopogonidae	250	131	119	99	49	30	72	132	118	138	112
	Tipulidae	95	70	25	39	16	16	24	49	46	58	37
	Dixidae	34	28	6	6	6	13	9	20	14	20	14
	Simuliidae	19	17	2	6	1	1	11	5	14	8	11
Ephemeroptera	Leptophlebiidae	50	47	3	7	21	5	17	12	38	9	41
	Ephemerellidae	48	34	14	17	8	14	9	14	34	22	26
	Baetidae	27	24	3	5	4	13	5	5	22	3	24
	Ameletidae	7	6	1	5	1	1	0	6	1	6	1
	Heptageniidae	7	5	2	0	2	4	1	3	4	6	1
Isopoda	Asellidae	202	181	21	66	40	47	49	121	81	60	142
Odonata	Gomphidae	15	15	0	2	2	4	7	10	5	3	12
	Cordulegastridae	3	2	1	1	2	0	0	3	0	3	0

## Appendix 3.2 (continued)

Plecoptera	Leuctridae	3617	2494	1123	845	1027	566	1179	2003	1614	296	3321
	Peltoperlidae	1065	960	105	214	401	173	277	573	492	203	862
	Nemouridae	600	411	189	139	141	135	185	240	360	222	378
	Perlodidae	88	78	10	22	24	20	22	39	49	32	56
	Chloroperlidae	63	59	4	12	43	4	4	49	14	22	41
	Perlidae	1	0	1	0	0	0	1	1	0	1	0
Trichoptera	Hydropsychidae	169	160	9	27	57	32	53	60	109	43	126
	Lepidostomatidae	115	108	7	23	39	21	32	35	80	11	104
	Rhyacophilidae	46	35	11	13	9	11	13	30	16	16	30
	Limnephilidae	27	25	2	6	6	11	4	13	14	18	9
	Philopotamidae	19	18	1	4	0	1	14	18	1	1	18
	Uenoidae	4	2	2	1	0	2	1	3	1	0	4
	Goeridae	1	0	1	0	1	0	0	0	1	1	0

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## **Vita**

### **Educational Institutions Attended and Degrees Already Awarded**

Bachelor of Science in Biology, Mercyhurst College, May 2011

High School Degree, Penfield High School, June 2007

### **Professional Positions Held**

2011-Present - Research Assistant (M.S.), University of Kentucky, Lexington, KY

2011 - Farm Technician, Mercyhurst College, Erie, PA

2010 - Student Researcher, Oregon State University, Corvallis, OR

### **Scholastic and Professional Honors**

Gamma Sigma Delta, Honor Society for Agriculture, 2013 to present

President's Prize, Graduate Student Ten-Minute Paper Competition, Second Place (Oral),

Annual Meeting of the Entomological Society of America, November 11-14,

2012, Knoxville, TN.

First Place, Eco-Vision Photo Contest, Student Section Awards, Annual Meeting of the

Ecological Society of America, August 7-12, 2011, Austin, TX.

Eagle Scout Award, Troop 310, Fall 2007, Penfield, NY.

### **Professional Publications**

Gonzalez, N., DeBano, S.J., Kimoto, C., Taylor, R.V., Tubbesing, C., and Strohm, C.

2013. Native bees associated with isolated aspen stands in Pacific Northwest Bunchgrass Prairie. *Natural Areas Journal* 33: 374-383.

Tubbesing, C., **Strohm, C.**, DeBano, S.J., Gonzalez, N., Kimoto, C., and Taylor, R.V.

2014. Insect visitors and pollination ecology of Spalding's catchfly (*Silene spaldingii*) in the Zumwalt Prairie of northeastern Oregon. *Natural Areas Journal* 34.

## **Presentations**

### Oral

Strohm, C.J. and Rieske-Kinney, L.K. 2013. From canopies to streams: Consequences of hemlock woolly adelgid-induced forest changes for benthic invertebrates. Annual Meeting of the Entomological Society of America, November 10-13, Austin, TX.

Strohm, C.J. 2013. Litter colonization and breakdown in streams threatened by hemlock decline. UK Department of Entomology, M.S. Exit Seminar, October 31, Lexington, KY.

Strohm, C.J. and Rieske-Kinney, L.K. 2013. From canopies to streams: Effects of hemlock woolly adelgid-induced forest changes on benthic invertebrates. Southern Forest Insect Work Conference, July 23-26, New Orleans, LA.

Strohm, C.J. and Rieske-Kinney, L.K. 2013. Hemlock woolly adelgid-induced eastern hemlock mortality affects benthic invertebrate colonization of leaf litter in headwater streams. Conference on Invasion Biology, Ecology, and Management, April 2-4, Lexington, KY.



Strohm, C.J. and Rieske-Kinney, L.K. 2012. Litter source determines macroinvertebrate colonization in Appalachian headwater streams. Annual Meeting of the Entomological Society of America, November 11-14, Knoxville, TN.

Strohm, C.J. and Rieske-Kinney, L.K. 2012. Effects of riparian vegetation and litterfall on benthic macroinvertebrates occupying Appalachian headwater streams threatened by eastern hemlock loss. Ohio Valley Entomological Association Annual Forum, September 28, Cincinnati, OH.

Strohm, C.J. and Rieske-Kinney, L.K. 2012. Macroinvertebrate colonization is influenced by litter source in Appalachian headwater streams. Southern Forest Insect Work Conference, July 24-27, Charlottesville, VA.

Strohm, C.J. 2012. Indirect effects of an invader: The role of riparian litter in shaping benthic macroinvertebrate communities. UK Department of Entomology, M.S. Proposal Seminar, February 17, Lexington, KY.

#### Poster

Strohm, C.J. and Rieske-Kinney, L.K. 2013. Who's doing what? Colonization and breakdown of litter in streams threatened by hemlock decline. Conference on Invasion Biology, Ecology, and Management, April 2-4, Lexington, KY.

Rieske L.K., Dodd, L.E., Adkins, J.K., Graziosi, I., Nelson, L.A., Sprinkle, M.A., and Strohm, C.J. 2011. Research in Forest Entomology: Building a knowledge base to understand and protect our natural resources. Annual Meeting of the Entomological Society of America, November 13-16, Reno, NV.

Tubbesing, C., Strohm, C.J., DeBano, S.J., Gonzalez, N., Kimoto, C. and Taylor, R.V. 2011. All bees, no flies for Spalding's catchfly: Bumble bee pollination of a

threatened plant. Annual Meeting of the Ecological Society of America, August  
7-12, Austin, TX.

**Service**

Moderator, National Conference on Undergraduate Research, Lexington, KY, April 2014

Moderator, Conference on Invasion Biology, Ecology, and Management, Lexington, KY,  
April 2013

Judge, Fayette County Science Fair, Lexington, KY. February 2013 and 2014

Volunteer, once per month, Entomology Exhibit, Explorium of Lexington Children's  
Museum. September 2011 to December 2013

Social Committee Chair, H. Garman Entomology Club. September 2011 to present