In-Stream Hemlock Twig Breakdown and Effects of Reach-Scale Twig Additions on Appalachian Headwater Streams

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(ABSTRACT)

Eastern hemlock (*Tsuga canadensis*) is a prominent tree in the forests of eastern North America, where it commonly grows along headwater streams. It is experiencing widespread mortality due to infestations of an introduced insect, the hemlock woolly adelgid (*Adelges tsugae*). Eliminations of tree species are known to have ecosystem-level effects, and one consequence of hemlock death is a change in allochthonous inputs to headwater streams. I predicted that hemlock twigs' dendritic structure, abundance, and resistance to decay currently make them highly effective retainers of leaves in headwater streams, with consequences for nutrient uptake. To understand the role of hemlock twigs in streams and to compare their functions to those of a potential replacement species, I (1) quantified the decomposition and microbial colonization of twigs and (2) manipulated twig standing crops to quantify effects on leaf retention and nutrient uptake. Hemlock twigs provide a poor-quality substrate for microbial colonization and growth relative to birch (*Betula lenta*) twigs and are more resistant to breakdown than birch. Although hemlock twigs appear to be effective in retaining leaves, they do not substantially affect reach-scale uptake of ammonium, which is much more strongly influenced by the timing of leaf inputs. As hemlocks are eliminated, the ability of streams in the southern Appalachian Mountain to retain organic matter may be compromised, and reach-scale effects of microbial activity may be altered.

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INTRODUCTION

Forests of eastern North America are currently experiencing a pathogen-induced shift in species composition as native hemlocks are infested with and killed by the hemlock woolly adelgid (*Adelges tsugae*), an insect introduced from Japan. Adelgid-induced mortality has the potential to eliminate eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*) from most of their range (Ellison *et al.* 2005). In 2006, adelgid infestation ranged from southern Maine to northern Georgia and eastern Tennessee and was continuing to spread (U.S. Forest Service 2007). In the southern Appalachian Mountains, eastern hemlock grows in mesic valley bottoms (Whittaker 1956) and, with the exception of high-elevation spruces and firs, is the only conifer typically found near streams. Most trees occur within 50 m of a stream, and distance to stream is a stronger predictor of eastern hemlock abundance and basal area than is elevation, slope, aspect, or terrain shape index (Narayanaraj 2006).

The structure and function of headwater streams are heavily influenced by adjacent terrestrial vegetation. Characteristics of plant species influence stream light regimes, food supply and quality, geomorphology, nutrient dynamics, and discharge, any of which can be altered by changes in the identity of riparian vegetation. Along forested streams, trees provide shade and limit algal primary production, but supply the basis for secondary production in the form of falling leaves and wood.

Among southern Appalachian trees found along streams, eastern hemlock fills a unique ecohydrologic role because it transpires throughout the year with a major peak in early spring, before deciduous leaf-out (Ford and Vose 2007). Summer transpiration by hemlock is low compared to broad-leaved trees (Ford and Vose 2007). Streams flowing through hemlock forests are known to contain assemblages of insects that are distinct from and less drought-tolerant than those in streams flowing through hardwood forests (Snyder *et al*. 2002). Changes in streamflow patterns due to hemlock death are expected to include increased winter and spring discharge and increased diel variation in discharge (Ford and Vose 2007). These changes may have subtle effects within headwater streams, but high flows could contribute to downstream flooding in spring storms.

Loss of hemlock may cause increased insolation and decreased insulation of streams. Intact hemlock canopies shade streams throughout the winter and early spring, when deciduous leaves are absent, and may stabilize stream temperature (Snyder *et al*. 2002). If more light reaches streams, particularly in the spring, algal production could be stimulated (Mulholland *et al.* 2006). The extensive rhododendron understory in the southern Appalachians, however, may expand in response to the increased light and obscure any direct effects of light on streams (Ellison *et al*. 2005).

Epidemics of forest pathogens like hemlock woolly adelgid are disturbances that can effectively remove a single species from an otherwise intact forest and thus alter the timing, quality, and annual rate of wood and leaf inputs entering streams. Chestnut blight (*Cryphonectria parasitica*) caused the functional extinction (*sensu* Estes *et al*. 1989) of American chestnut (*Castanea dentata*) from eastern forests in the 20th century. As a result, chestnut leaves were eliminated as a food supply for stream organisms and replaced with lower-quality oak leaves (Smock and MacGregor 1988). The blight also affected inputs of wood to streams. American chestnut was one of the most abundant species represented in stream wood in the 1990s, despite the near-complete elimination of

living chestnut trees by the early 1940s (Hedman *et al*. 1996, Wallace *et al*. 2001). Chestnut logs are large and composed of decay-resistant wood, so they are expected to have long-lasting effects on streams.

Wood in streams slows and diversifies the flow of water, causing the accumulation of sediment and organic matter and modifying stream geomorphology (Bilby and Likens 1980, Bilby 1981). These processes in turn provide favorable conditions for the biotic processing of leaves, wood, and dissolved nutrients (Bilby and Likens 1980, Valett *et al.* 1996, Hedin *et al.* 1990). Compared to leaves, which often make up the largest fraction of allochthonous inputs to streams, wood breaks down slowly and makes a small direct contribution to stream energy flow. Factors such as species, particle size, and external nutrient supply affect the microbial colonization and the subsequent breakdown of wood. Gymnosperms such as hemlock decompose more slowly than angiosperms, a relationship that is often explained by the high lignin content and high C:N ratio of gymnosperm wood (Harmon *et al.* 1986). In addition to these qualities, gymnosperm wood contains less nutrient-rich living tissue than angiosperm wood and has smaller and less continuous water-conducting tissue than angiosperms (Harmon *et al*. 1986). The diameter of vascular tissue, which varies among angiosperms as well, can limit penetration by fungi, which, along with bacteria (particularly actinomycetes) and macroinvertebrates, are important decomposers of wood (Harmon *et al*. 1986). Secondary compounds, abundant in gymnosperm tissue and in the heartwood of many species, can be especially limiting to decomposition of wood by bacteria and fungi because they inhibit oxidative enzyme function (*e.g.*, Ward *et al*. 1997).

Hemlock needles made up 17% of leaf fall entering eight southern Appalachian stream reaches in one year, and large wood in these streams ranged from 0% to 70% hemlock (K. Morkeski, J. R. Webster, and E. F. Benfield, unpublished). In eleven 500-m reaches running through forests in a range of seral stages, hemlocks represented 36% of wood volume and made up 61% in old-growth stands (Hedman *et al*. 1996). Adelgid-induced tree mortality is likely to cause initial increases and eventual declines in quantities of hemlock needles and wood in streams. Needles will be rapidly lost from trees as infestation progresses. Inputs of needles will then be replaced by leaves of rhododendron or, initially, early successional species such as sweet birch (*Betula lenta*) and, subsequently, other forest trees (Ellison *et al*. 2005, Eschtruth *et al.* 2006, Orwig and Foster 1998). As trees die, their trunks and branches will fall into streams over a period of years to decades. Because they are large gymnosperms with a high tannin content, hemlocks in streams will decay very slowly, like chestnut wood (Hedman *et al*. 1996, Harmon *et al*. 1986). Hedman *et al*. (1996) predicted that carry-over hemlock wood (*i.e.*, wood from a stand of hemlocks replaced by other species) in southern Appalachian streams would last for about 200 years.

Small hemlock wood, like large wood, is abundant in streams. In seven southern Appalachian headwater streams, hemlock sticks made up at least 16% of wood under 5 cm in diameter (K. Morkeski, J. R. Webster, and E. F. Benfield, unpublished). Organic matter accumulations, defined as leaves and sticks < 5 cm in diameter, are the most common morphological features in small forested streams of the southern Appalachians (Golladay *et al*. 1987). I hypothesized that hemlock twigs are very effective retainers of leaves in headwater streams because they are highly branched, abundant, and resistant to decay. Leaf detritus and its associated microbial decomposers are known to drive

demand for nutrients (*e.g*., Tank *et al*. 2000), so any structure or process that increases the abundance, size, or longevity of organic matter accumulations has the potential to influence uptake and downstream availability of nutrients. I speculated that if hemlock twigs contribute substantially to leaf retention, they will increase in-stream nutrient uptake.

The objectives of this project were to understand the role of hemlock twigs in streams and to compare the functions of hemlock twigs with twigs of a potential replacement species, sweet birch. To accomplish those goals, my approach involved (1) quantifying the breakdown and microbial colonization of twigs and (2) manipulating twig standing crops to assess effects of hemlock and birch twigs on leaf retention and nutrient uptake.

METHODS

Study Sites

This study was conducted at Coweeta Hydrologic Laboratory in western North Carolina, U.S.A. The stream reaches used included the east fork of Camprock Branch (Watershed 10) and a section of Little Hurricane Branch just downstream of Watershed 3. Watershed 10 was logged between 1942 and 1956 and has since been allowed to return to mixed hardwood forest. Watershed 3 was completely cleared in 1940, was used as an experimental farm for the next twelve years, and was then planted in white pine and tulip poplar. The segments of Little Hurricane Branch used in this study are located downstream of the white pine area, and riparian vegetation consists of early-successional Appalachian cove forest. Little Hurricane Branch and the east fork of Camprock Branch are each first-order streams with similar discharge, gradient, and aspect (Table 1). *T. canadensis* and *B. lenta* occur along both streams, and other prominent species include *Rhododendron maximum* (great rhododendron), *Liriodendron tulipifera* (tulip poplar), and *Quercus prinus* (chestnut oak).

Table 1. Site characteristics. Discharge and conductance values are averages of data collected on five occasions during the study period. NH_4-N concentration refers to the background concentration of nitrogen as ammonium.

	Camprock Branch	Little Hurricane Branch
Elevation	830 m	730 m
Aspect	S	SSE
Gradient	9 cm/m	16 cm/m
Discharge	4.5 L/s	3.9 L/s
NH_4-N concentration		
October	0.49 µg/L	0.13 µg/L
March	4.9 µg/L	4.7 µg/L
Specific conductance	15.2 µS	45.0 µS

Twig Preparation

Hemlock twigs were collected in July 2005 from trees that were felled after being killed by the hemlock woolly adelgid. They were removed from logging slash piles less than two weeks after trees were cut down. Birch twigs were collected from live saplings in July 2005. Twigs of both species were air-dried in the laboratory, attached birch leaves were removed by hand, and twigs were trimmed to a maximum diameter of 3 mm.

Twig Decay Properties

Bundles of twigs (average mass 6.3 g) were each weighed, bound with a plastic zip-tie, and labeled with an identification tag. In September 2005, bundles (n = 42 per

stream) were placed in Camprock Branch and Little Hurricane Branch and anchored in place. On seven dates, three hemlock bundles and three birch bundles were retrieved from each stream. Immediately after the collections, twig bundle wet weights were recorded, one subsample from each bundle was removed for microbial respiration assays, and one subsample was removed for measurement of fungal biomass. Each subsample for respiration or fungal analysis consisted of several short twig segments with an approximate combined surface area of 7.5 cm^2. Subsamples for respiration were placed in vials of stream water and kept on ice until returned to the laboratory at Virginia Tech. Subsamples for fungal biomass were placed in polypropylene centrifuge tubes containing 5 mL methanol, kept on ice until returned to the lab, and then refrigerated at 4 ºC until processed.

Microbial respiration was measured as the consumption of dissolved oxygen in flasks containing water and twig segments in a procedure similar to the one used by Chaffin *et al.* (2005) for leaves. Within 24 h of return to the laboratory, twig samples designated for microbial respiration assays were placed in 30-mL Erlenmeyer flasks that were subsequently filled with stream water and stoppered. For each sample, the source of water matched the stream from which the twigs were taken. On five dates, oxygen concentration was determined by Winkler titration (American Public Health Association 1998), and in November, oxygen concentration was determined by dissolved oxygen miniprobe (Model ISO2 DO meter and OXEL-P probe, World Precision Instruments, Inc., Sarasota, Florida). On dates when Winkler titrations were used, six additional flasks containing no twigs were filled with water from each stream. Three of these samples were immediately fixed to determine initial dissolved oxygen concentration, and three were fixed following an overnight incubation period during which twigs and stream water were kept at approximate stream temperature. In November, the same incubation procedure was used, but the oxygen concentration in each flask containing twigs and in six flasks containing only stream water was directly measured at the beginning and end of the incubation period. The oxygen consumption in flasks containing only water was subtracted from the oxygen consumption in flasks containing twigs and water to determine the microbial oxygen consumption on twigs. After the final measurements of oxygen concentration, all twig segments were dried at 60 ºC for 48 h and weighed to determine their dry mass. Dry mass was converted to ash-free dry mass (AFDM) using conversion factors obtained in determining breakdown rate (below), so that the units for respiration were $\mu g\ O_2\ g^{-1}$ AFDM h^{-1}.

Fungal biomass in twig tissue was determined by extraction of ergosterol, a lipid found exclusively in fungal cell membranes, using the method described by Newell *et al*. (1988). Twig segments placed in methanol were heated to 65 ºC for two hours to remove ergosterol, which was then saponified with potassium hydroxide, extracted into pentane, purified by evaporation, and redissolved into methanol. Concentrations were then determined using high-performance liquid chromatography. Ergosterol was converted to fungal biomass using a value of 5.5 mg ergosterol/g C, the average from 14 aquatic hyphomycete strains as determined by Gessner and Chauvet (1993). For both respiration rate and fungal biomass, differences between hemlock and birch were assessed using paired t-tests of means for each species (n=6). Data for each species were paired by date to account for variation through time.

Birch and hemlock twig breakdown rates were determined through measurements of the twig bundle mass remaining at sequential collections. After bundles were collected and subsamples were removed for respiration and ergosterol analysis, they were dried at 60 ºC and weighed. The total dry mass of a bundle consisted of this measurement plus the dry mass of the respiration and ergosterol samples. Bundles were ground, and a portion of the ground material was ashed at 550 °C for 1 h to determine the AFDM of each bundle. This value was compared to the initial mass of the bundle, which was converted from air-dry mass to AFDM using the percent organic matter of a set of bundles that was never incubated in streams. The breakdown rate of each species was calculated as the inverse slope of the linear regression between the natural log of the percent AFDM remaining and the time of in-stream incubation (Benfield 2006). Loss of mass was determined to be significant if the slope of the regression was significantly different from zero. A two-way analysis of variance (ANOVA) with time and species as factors was used to determine whether birch and hemlock lost mass differently. Percent bundle mass remaining at the time of collection was natural log-transformed.

Reach-Scale Dynamics

In September 2005, 2170 g birch twigs were added to a 50-m reach of Camprock Branch, and 2220 g hemlock twigs were added to a 50-m reach of Little Hurricane Branch. In both reaches, which were located just upstream of the twig bundle incubation sites, sticks were added to attain a standing crop of 40 g/m^2, which represents a four-fold increase over the standing crop of hemlock sticks measured in seven reference reaches at Coweeta in March 2005 (K. Morkeski, J. R. Webster, and E. F. Benfield, unpublished). Twigs were scattered evenly across the area of the each stream reach and were not anchored in any way. Ammonium uptake, leaf retention, and coarse benthic organic matter (CBOM) standing crops were assessed in each treatment reach and in upstream reference reaches. Measurements were completed once before twig additions, in September 2005, and four times after twig additions, in October 2005, November 2005, January 2006, and March 2006.

The transport of leaf analogs was used as an indicator of the leaf retention capacity of each reach, based on the method used by Webster *et al.* (1994). In September, October, January, and March, 25 leaf analogs consisting of triangles (10.5 x 7.0 x 12.5 cm) of Rite-in-the-Rain paper (J.R. Darling Co., Tacoma, Washington) that had been soaked overnight in water were dropped into the reaches. After 1-2 h, the distance traveled by each triangle was measured.

CBOM standing crops were quantified by collecting fifteen samples (with the exception of October 2005, when ten samples were taken) with a 30 cm-diameter stovepipe corer. Samples were dried at 60 °C and stored at room temperature until processed. They were sorted by organic matter type (leaves, birch sticks, hemlock sticks, other sticks, bark, and fruit), and weighed. For each reach and date, all organic matter of one type was combined and ground. Three to five 0.25-g subsamples were removed and ashed at 550 °C for 1 h. The percent of the subsamples combusted was multiplied by the total dry mass to determine the ash-free dry mass (AFDM) per m^2. Differences in standing crops between reference and treatment reaches was assessed using paired t-tests of mean standing crops in each reach (n=10 in October and n=15 on other dates). Data

from each reference reach and its treatment reach were paired for each date to account for variation through time.

Ammonium uptake was measured using short term (ca. 3 h) enrichments during which the stream NH_4-N concentration was elevated by approximately 50 µg/L and a conservative tracer (chloride) was simultaneously added (Webster and Valett 2006). Because a stable isotope tracer method or the multiple-enrichment method of Payn *et al*. (2005) was not used, measurements of uptake are most valuable in comparison to each other and not as absolute estimates. Before the co-injections began, a water sample for NH_4-N analysis was collected and specific conductance was measured with a conductivity meter (YSI Model 30, YSI, Inc., Yellow Springs, Ohio) at each of five locations within each reach. Addition of ammonium-chloride solution to the stream reach at a constant rate was begun after background sampling was completed. When the addition of chloride had elevated downstream conductance to a constant level, three water samples and one conductance measurement were collected at each of the locations used for background samples. Water samples were frozen until NH_4-N concentration was measured using USEPA Standard Method 349.0 (Zhang *et al*. 1997) on a Technicon II continuous flow analyzer (Technicon Industrial Systems, Tarrytown, New York) for September, October, and November samples or a Lachat QuikChem 8500 flow injection analyzer (Hach, Loveland, Colorado) for January and March samples.

To calculate ammonium demand, the plateau concentrations were corrected for background ammonium concentration and for the effect of dilution, that is, the increase in ammonium concentration from background to plateau was divided by the increase in conductance from background to plateau. The resulting values were natural log-transformed and subjected to linear regression analysis with distance downstream as the independent variable. The inverse slope of the regression was taken as the uptake length, S_w (Webster and Valett 2006). Uptake velocity, V_f, which is a measure of how quickly a nutrient moves from the water column to the benthos, and as such normalizes for hydrologic variation and indicates uptake relative to the availability of the nutrient, was calculated using the equation

$$V_f = Q * 1/S_w * x * 60$$

where Q is stream discharge (L/s), x is average stream width (m), and 60 is a conversion factor used to bring V_f into units of mm/min (Webster and Valett 2006). The product of uptake velocity and background concentration is uptake, the flux of NH_4-N moving from the water column to the benthos per unit area of streambed.

RESULTS

Twig Decay Properties

Fungal biomass on twigs incubated in streams ranged from 2.00 to 8.34 mg C/g AFDM on birch (mean 5.00 mg/g AFDM) and 2.25 to 5.81 mg/g AFDM on hemlock (mean 3.80 mg/g AFDM) (Fig. 1). At the first two sampling dates, birch and hemlock twigs contained statistically indistinguishable amounts of fungal biomass (t-tests for 1 and 30 Oct., p=0.787 and p=0.304), but, beginning in November, hemlock twigs

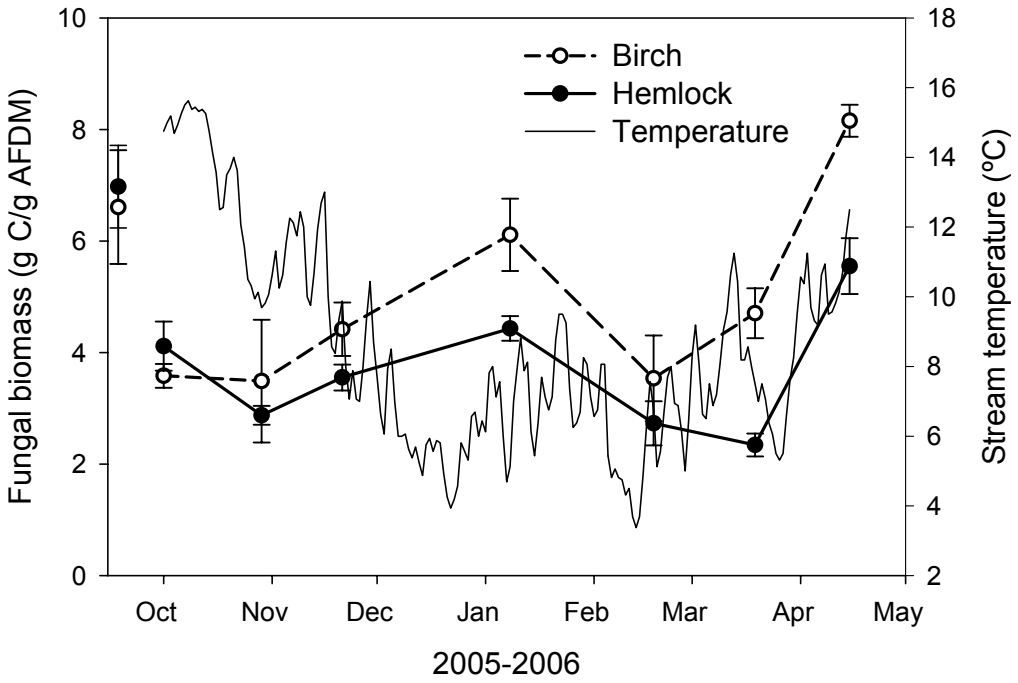

Figure 1. Fungal biomass on birch twigs (dashed line, open symbols) and hemlock twigs (solid line, closed symbols) and average daily water temperature in Camprock Branch (solid line, no symbols) measured from September 2005 to April 2006. Each symbol represents a mean (n = 6) ± 1 SE. Fungal biomass prior to in-stream incubation is indicated by the disconnected symbols.

contained less fungal biomass than birch twigs (paired t-test of means (n = 6) from six dates, $p<0.05$) Before being incubated in streams, birch twigs contained 6.78 mg fungal C/g AFDM, and hemlock twigs contained 7.07 mg fungal C/g AFDM, indicating that colonization of both species by terrestrial fungi had occurred.

Microbial respiration rates by species ranged from 3 to 24 μg O_2 g^{-1} AFDM h^{-1} for hemlock and 12 to 54 μg O_2 g^{-1} AFDM h^{-1} for birch (means of six bundles) (Fig. 2). Hemlock twigs consistently supported lower respiration rates than birch twigs (paired t-test of means (n = 6) on six dates, $p<0.05$). Both respiration rate and fungal biomass

fluctuated over the seven months of study and appeared to be influenced by stream temperature.

The breakdown rates of twigs were 1.17 y^{-1} (95% confidence interval: 0.850-1.486 y^{-1}) for birch and 0.402 y^{-1} ^{1} (95% confidence interval: 0.264-0.568 y^{-1}) for hemlock. Both species experienced significant loss of mass. At the end of the 210-day incubation period, 30-56% of birch stick mass and 60-80% of hemlock stick mass remained (Fig. 3). A significant interaction occurred between stream, species, and time, indicating that breakdown progressed differently in the two streams, but that the effect of the streams was not the same for both species. Breakdown rates for both hemlock and birch were slightly lower in Camprock Branch than in Little Hurricane Branch.

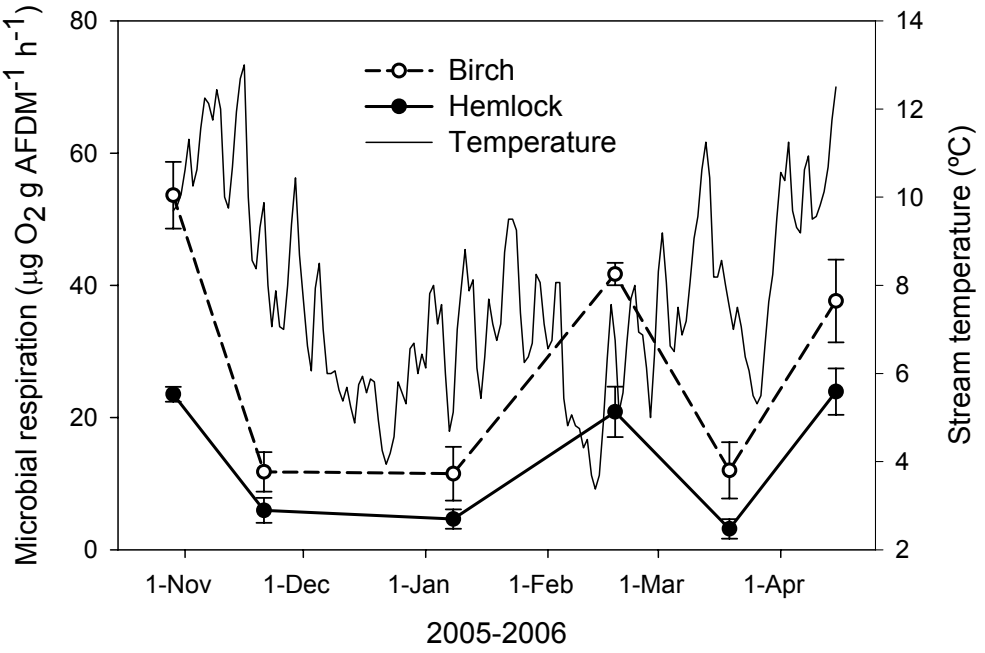

Figure 2. Microbial respiration rates on birch twigs (dashed line, open symbols) and hemlock twigs (solid line, closed symbols) and average daily water temperature in Camprock Branch (solid line, no symbols) measured from October 2005 to April 2006. Each symbol represents a mean (n =6) ± 1 SE.

Reach-Scale Dynamics: Organic Matter

Leaf analog transport distance was very short in every instance that it was measured. The maximum distance traveled by a leaf analog was 130 cm. No trends in leaf transport were observed among stream reaches or among sampling dates, and leaf transport was excluded from further analysis.

Total CBOM standing crops were lowest in September in all reaches and increased and subsequently decreased from that point (Fig. 4). Peak concentrations were similar (approximately 250 g AFDM/m^2) in both treatment reaches, but they occurred at

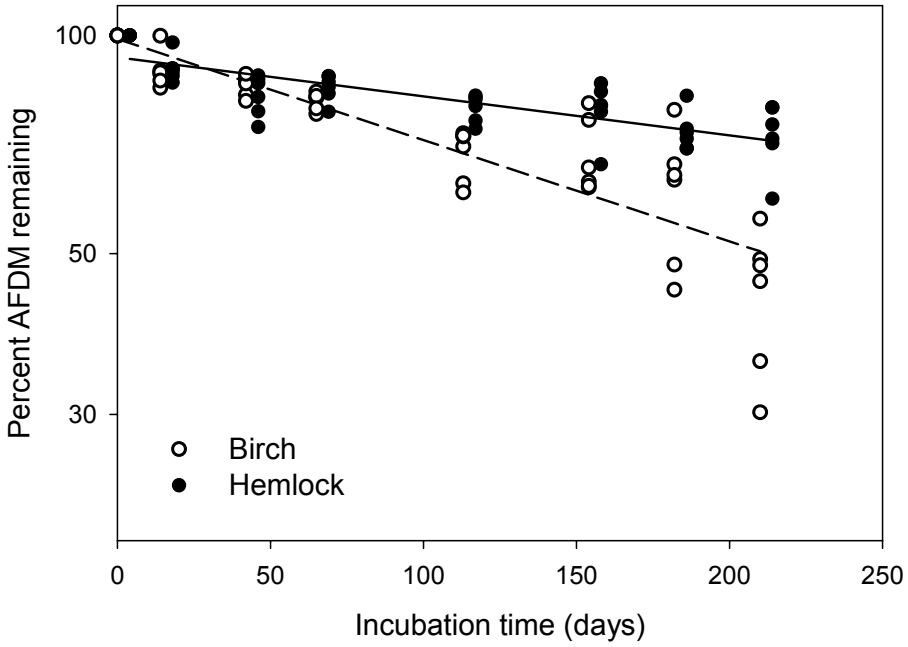

Figure 3. Percent ash-free dry mass of hemlock and birch sticks remaining over 210 d of incubation in two streams. Open symbols are birch twig bundles. Closed symbols are hemlock twig bundles and are slightly offset to avoid obscuring birch symbols.

different times. CBOM was most abundant in Little Hurricane Branch in October and most abundant in Camprock Branch in January. The reference reaches followed the same temporal patterns as their respective treatment reaches. CBOM standing crops were significantly higher in the hemlock addition reach (Little Hurricane Branch) than in its reference reach (paired t-test of means on five dates, $p<0.05$). Although CBOM standing crops were slightly larger in the birch addition reach than in its reference reach in October, November, and January, this difference was not statistically significant (paired t-test of means on five dates, $p=0.101$, and on three dates, $p=0.068$).

Much of the difference that existed between treatment and reference reaches was due to the addition of twigs. As intended, the addition of twigs increased the standing crops of twigs in the treatment reaches. Five weeks post-addition, the hemlock treatment reach contained 19.8 g hemlock twigs/m^2, and its upstream reference reach contained 0.7 g/hemlock twigs/m^2 (Table 2). At the same time, the birch reference reach contained no twigs identified as birch, while the treatment reach contained 26.7 g birch twigs/m^2. In collections after October, the amount of birch twigs in the treatment reach declined, and no birch twigs were ever found in the reference reach. Hemlock twigs in the hemlock treatment reach initially declined and then increased slightly over the course of the study, which may reflect the patchy distribution of sticks over the stream bottom or inputs of twigs from hemlock trees near the stream.

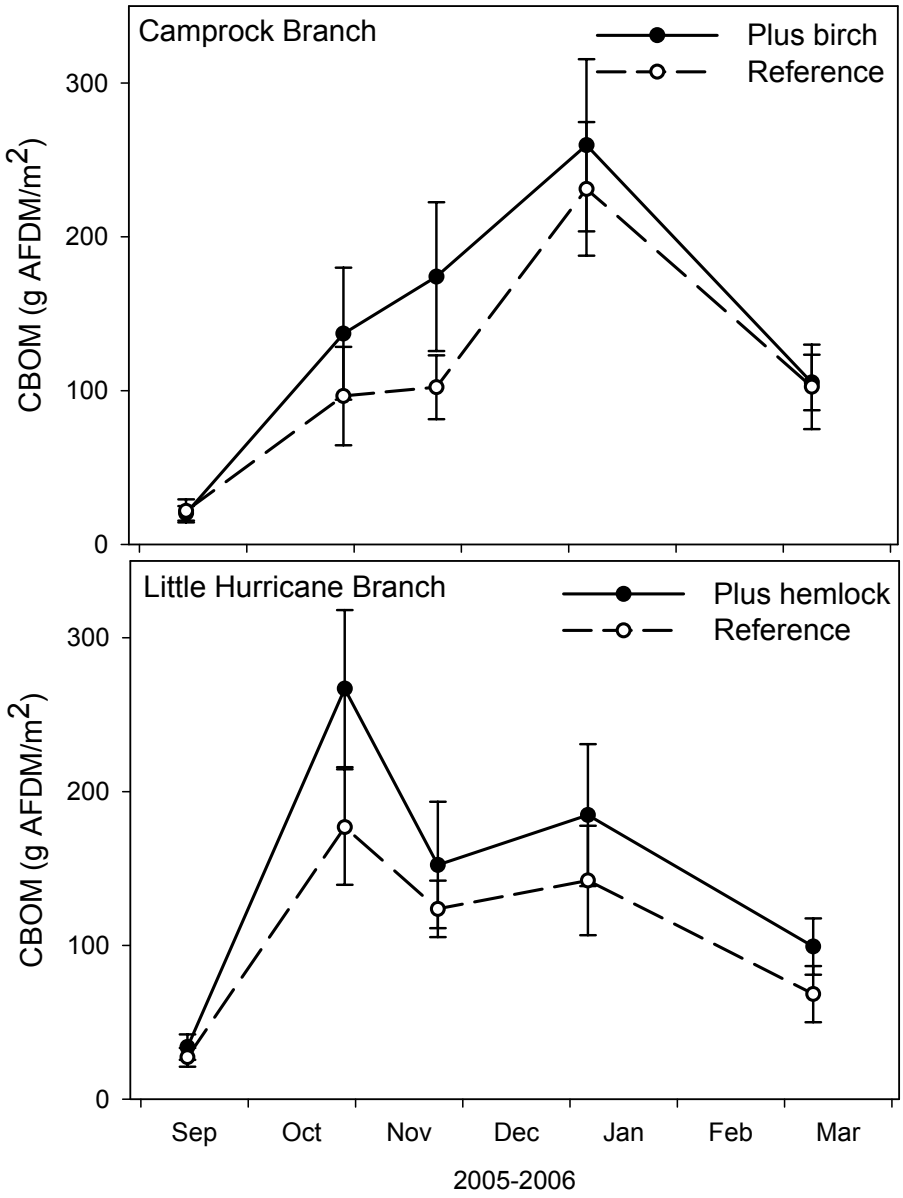

Figure 4. Standing crops of coarse benthic organic matter (CBOM) in Camprock Branch, with plots for the birch twig addition reach and its reference reach, and Little Hurricane Branch, showing the hemlock twig addition reach and its reference reach. Error bars = ± 1 SE.

The mass of small wood not identified as birch or hemlock was relatively steady in all reaches through the September, October, and November collections (mean 15.0 g/m^2) and increased sharply by January (mean 55.7 g/m^2). By March, the mass of sticks was intermediate to the fall and winter values (35.0 g/m^2). The additions of birch and hemlock sticks approximately doubled the standing crop of sticks in the treatment reaches in October, but were only a quarter of the mass of other sticks in January. While the added sticks did increase the small wood standing crops in the treatment reaches, their mass did not exceed the maximum mass of all other species.

Table 2. Standing crops (g AFDM/m^2) ± 1 SE of hemlock sticks, birch sticks, and other sticks in the twig addition (treatment) and reference reaches of Little Hurricane Branch and Camprock Branch.

		September	October	November	January	March
Little Hurricane Branch						
Hemlock sticks						
	Treatment	0.0 ± 0.0	19.8 ± 5.8	12.4 ± 7.2	14.8 ± 4.6	22.4 ± 8.0
	Reference	1.0 ± 1.0	0.7 ± 0.5	1.4 ± 1.2	0.8 ± 0.5	2.3 ± 1.0
Other sticks						
	Treatment	12.1 ± 4.9	19.2 ± 7.3	13.9 ± 3.4	40.9 ± 10.0	36.0 ± 8.8
	Reference	16.2 ± 4.1	12.0 ± 4.1	15.0 ± 4.4	63.9 ± 20.8	52.7 ± 15.3
Camprock Branch						
Birch sticks						
	Treatment	0.0 ± 0.0	26.7 ± 8.4	16.9 ± 5.0	13.4 ± 4.6	12.2 ± 5.8
	Reference	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other sticks						
	Treatment	10.1 ± 2.8	19.4 ± 7.6	18.4 ± 7.8	63.8 ± 21.4	22.0 ± 6.7
	Reference	11.9 ± 3.4	15.3 ± 7.1	16.1 ± 4.4	54.4 ± 11.2	29.4 ± 11.1

Bark and miscellaneous organic matter typically made small contributions to benthic organic matter standing crops (0-6 g ADFM/m^2), but both treatment reaches did contain slightly more miscellaneous organic matter than their reference reaches. The treatment reach of Camprock Branch contained notably more miscellaneous organic matter than the other reaches on two dates, which was probably due to the inclusion of acorns in the samples. When the treatment reach of Little Hurricane Branch contained slightly more miscellaneous organic matter than its reference reach (in January), the difference was due to the presence of pine needles in the treatment reach.

The remaining difference between the CBOM standing crop treatment and reference reaches of Little Hurricane Branch was due to the significantly larger leaf standing crop in the treatment reach (paired t-test of means on five dates, $p<0.05$) (Fig. 5a). Leaf standing crops in the reference and treatment reaches of Camprock Branch were not significantly different from each other (paired t-test of means on five dates, $p=0.654$) (Fig. 5b), suggesting that, relative to birch twigs, hemlock twigs were more effective retainers of leaves. Like CBOM standing crops, leaf standing crops showed peaks at distinctly different times. In Little Hurricane Branch, leaf mass peaked in October, while in Camprock Branch, leaf mass peaked in January.

Reach-Scale Dynamics: Ammonium Uptake

NH_4-N uptake lengths ranged from 12.6 to 92.6 m and generally followed a seasonal pattern of long uptake lengths in the summer, decreasing uptake lengths during autumn leaf fall, and increasing uptake lengths into the spring (Fig. 6). In September, before any twigs were added to the streams, uptake lengths in the two reference reaches

were long (74.1 m in Camprock Branch and 92.6 m in Little Hurricane Branch). Uptake length in the treatment reach of Camprock Branch at the same time was only 18.8 m, while no significant uptake was evident in the treatment reach of Little Hurricane Branch. Uptake lengths in all reaches were short (12.4-20.3 m) in October and November and increased by January in Camprock Branch and by March in Little Hurricane Branch.

Uptake velocity ranged from 0 mm/min (assumed for the reach with no significant uptake) to 24.0 mm/min (Fig. 7). Uptake velocity in the two reference reaches was very similar, with low values in September (1.41 and 3.32 mm/min), high values in October and November (15.2-18.5 mm/min), and intermediate values in January and March (3.74-

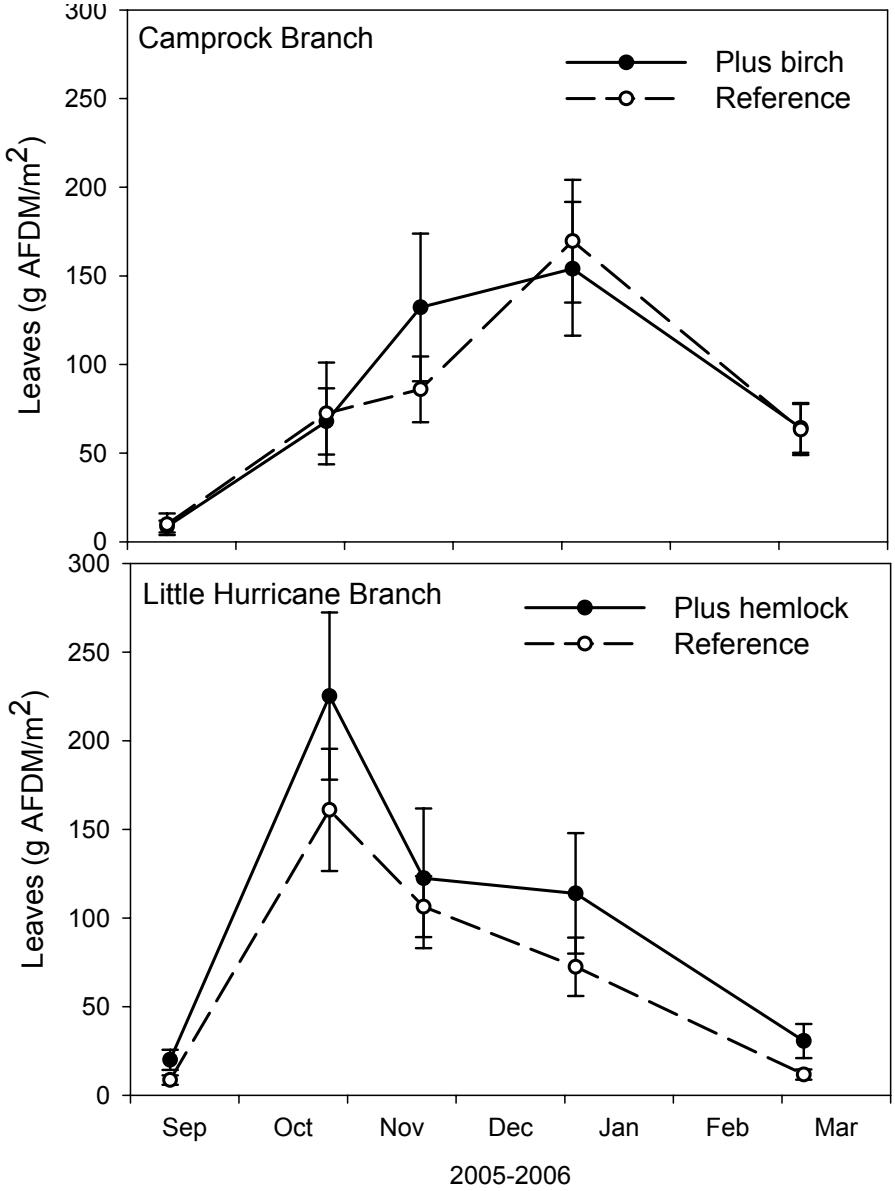

Figure 5. Standing crops of leaves in Camprock Branch, with plots for the birch twig addition reach and its reference reach, and Little Hurricane Branch, showing the hemlock twig addition reach and its reference reach. Error bars = ± 1 SE.

9.37 mm/min). Uptake velocities in the hemlock addition reach were similar to those in the reference reaches in September, October, and March, but, in contrast to the reference reaches, included a drop in November and a peak in January. The birch addition reach followed a unique pattern. Uptake velocity there was higher than V_f in the other three reaches in September (before twigs were added) and dropped to a lower value in October. In November, the same reach produced the highest V_f measured in this study, but by January and March, its uptake velocities were less than or equal to those in the reference reaches. Uptake velocity was significantly related to leaf standing crops in the hemlock

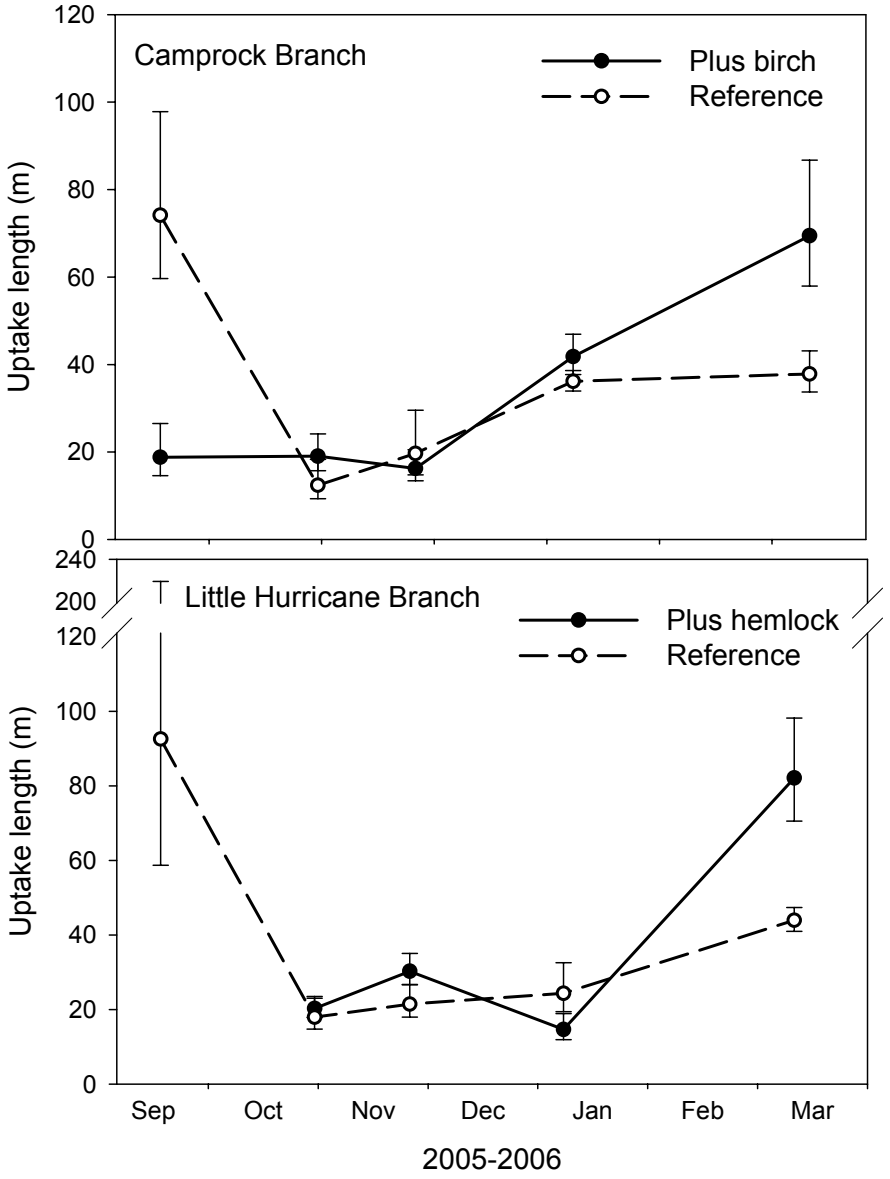

Figure 6. Ammonium uptake lengths in Camprock Branch, with plots for the birch twig addition reach and its reference reach, and Little Hurricane Branch, showing the hemlock twig addition reach and its reference reach. Error bars represent 95% confidence limits.

addition reach and its reference ($r^2 = 0.607$, $p<0.05$, Fig. 8) and in all four reaches when assessed together ($r^2 = 0.305$, $p<0.05$), but not for the birch addition reach and its reference alone.

Uptake of NH_4-N followed many of the same patterns as uptake velocity but showed more similarity between the two reference reaches and sharper peaks in the treatment reaches (Fig. 9).

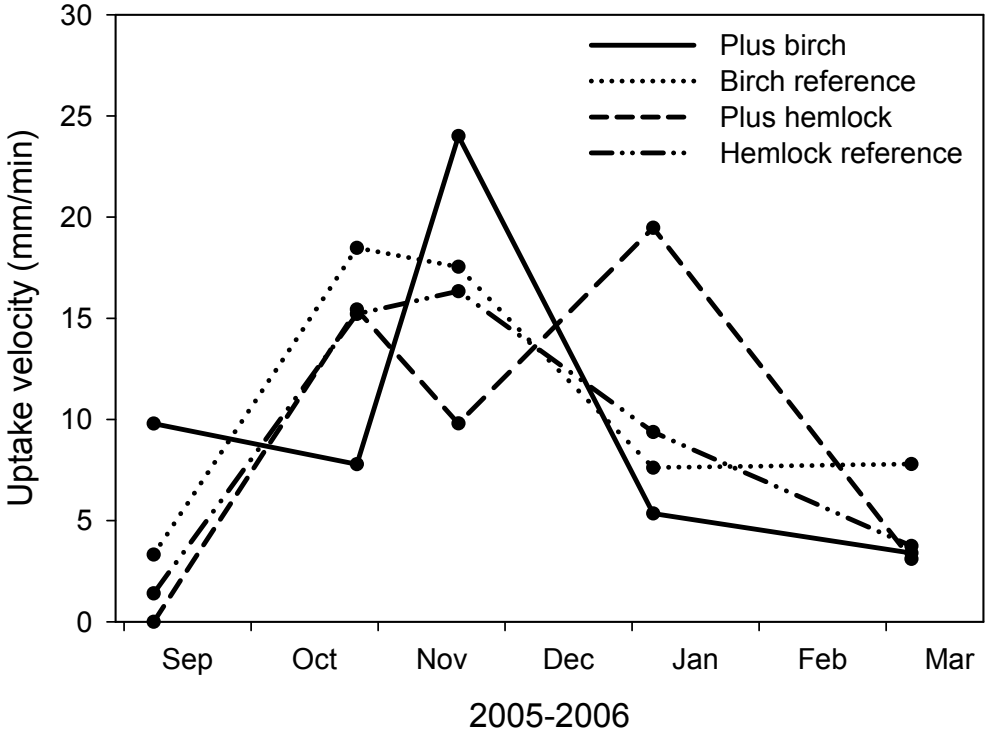

Figure 7. Ammonium uptake velocity in the four stream reaches.

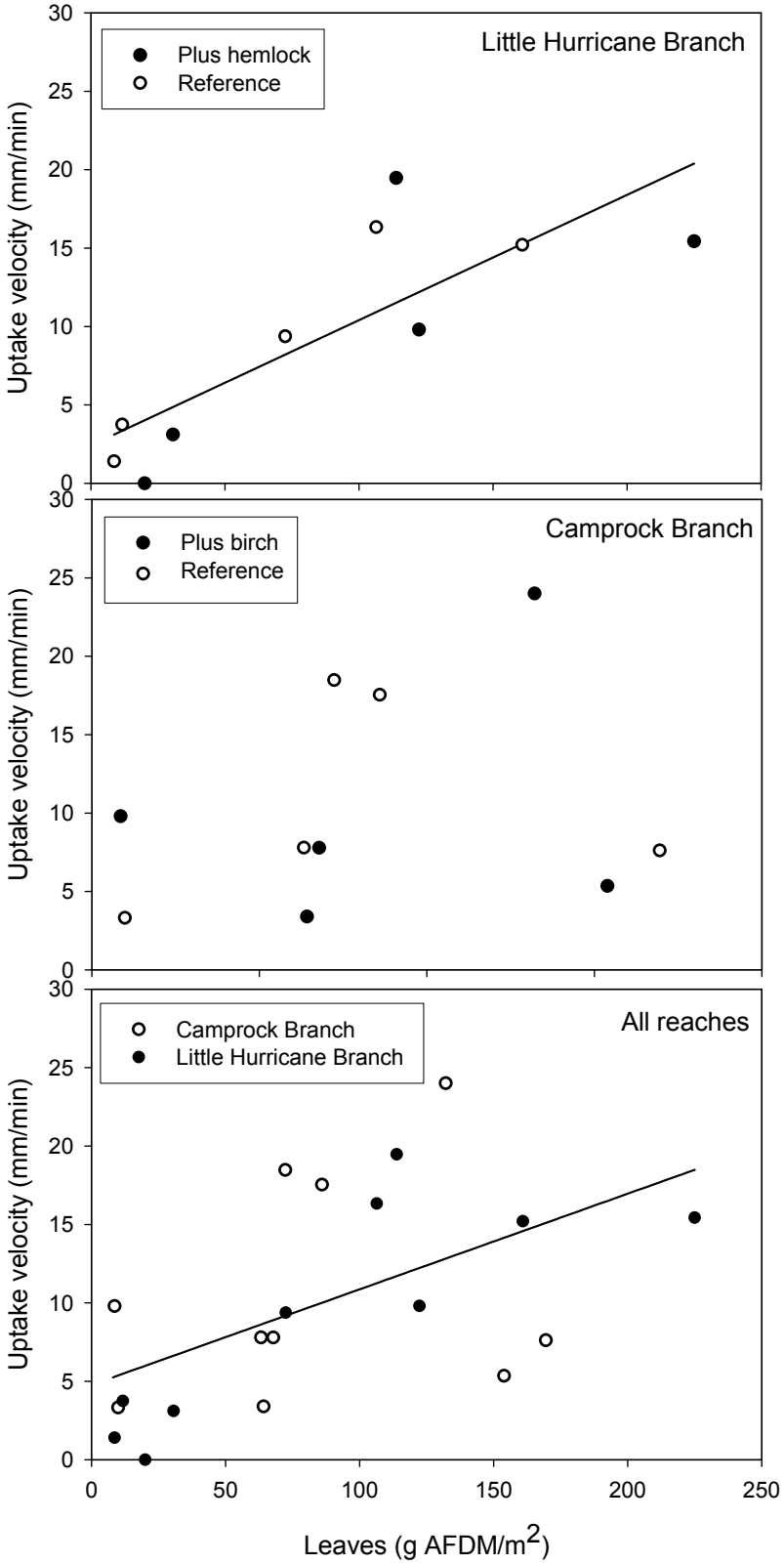

Figure 8. Relationships between leaf standing crop and ammonium uptake velocity in Little Hurricane Branch, Camprock Branch, and both streams.

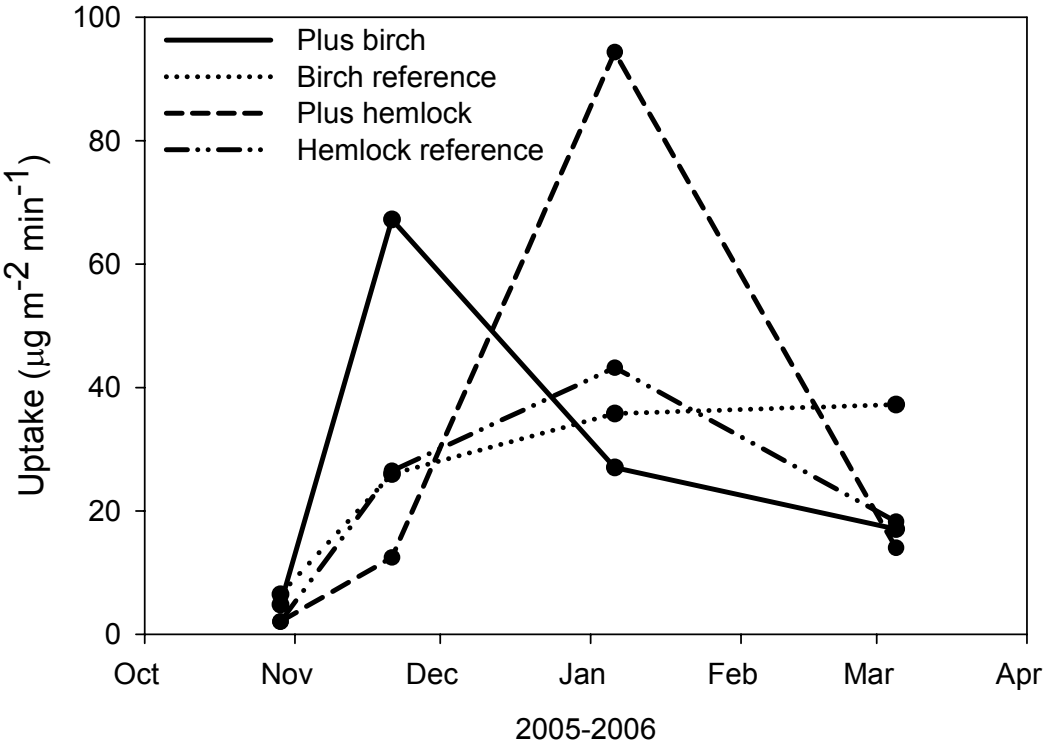

Figure 9. Areal ammonium uptake in the four stream reaches.

DISCUSSION

Twig Decay Properties

Birch twigs supported more fungal biomass (Fig. 1) and higher microbial respiration rates than hemlock sticks (Fig. 2), and they broke down more quickly than hemlock twigs (Fig. 3). Taken together, my results show that small wood from hemlock trees is a low-quality microbial substrate and is long-lasting relative to wood of sweet birch, one of the species that replaces adelgid-killed hemlocks (Eschtruth *et al*. 2006, Orwig and Foster 1998). Hemlock death will result in the elimination of small and large hemlock wood as a short-term and long-term structural component of streams and possibly as an important food source. The turnover time, as the inverse of the breakdown rate, for hemlock twigs is 2.5 y, while the turnover time for birch twigs is < 1 y. The longer turnover time for hemlock suggests that hemlock twigs are likely to be abundant relative to other forms of organic matter in the summer, when leaves are scarce in Coweeta streams, and could be a primary support for microbes and insects at this time.

In one of the few studies aside from this one to measure fungal biomass on non-comercially-processed sticks, Findlay *et al*. (2002) sampled small wood in 9 headwater streams across the U.S. Among all streams, they found approximately 5 mg fungal C/g AFDM, which is identical to the average on stream-incubated birch twigs in this study. At Ball Creek, a stream at Coweeta, Findlay *et al*. (2002) found approximately 3 mg fungal C/g AFDM, which is similar to the average biomass on stream-incubated hemlock twigs in my study. Díez *et al*. (2002) also used segments of branches (3 cm diameter). The sticks were incubated within the Agüera catchment, Spain, in streams that are warmer and have higher nutrient concentrations than Coweeta streams. These differences in environmental conditions may explain why fungal biomass on alder and eucalyptus sticks (peak values of 25 and 16 mg/g, respectively) exceeded the amounts I found (maximum 9.33 mg/g). However, fungal biomass on pine sticks in the Agüera catchment (peaks of 4.2-6.5 mg/g) was similar to and even slightly lower than the fungal biomass I found on hemlock sticks (maximum 7.59 mg/g). These findings support the idea that microbial colonization and decomposition of organic matter in aquatic environments can be limited by the quality of the organic substrate (e.g., Webster and Benfield 1986, Harmon *et al*. 1986), the supply of nutrients from the water column (Golladay and Webster 1988, Suberkropp and Chauvet 1995, Tank and Dodds 2003, Stelzer *et al*. 2003, Gulis *et al*. 2004, Aumen *et al*. 1983), or both (Spänhoff and Meyer 2004, Tank and Webster 1998). Fungal biomass on pine sticks 2.5-7.8 cm in diameter incubated for up to 56 weeks in a sandy lowland stream in Germany was 7-13 mg fungal C/g dry mass on bark and 0.9-2.2 mg fungal C/g dry mass on wood (Spänhoff and Gessner 2007; values calculated from ergosterol concentrations and the ergosterol-to-biomass conversion factor of Gessner and Chauvet 1993). Biomass on hemlock and birch twigs was intermediate to these values, which is not surprising considering that the twigs consisted of roughly equivalent portions of bark and wood.

Other investigators have used veneers, dowels, or popsicle sticks without bark in quantifying fungi on wood. A major advantage of this technique is the ease of expressing biomass on a per-area basis. Aquatic fungi primarily colonize the surface of wood, so wood surface area is considered to be a better basis for measurement than wood mass

(Tank and Webster 1998). To compare measurements of fungal biomass in this study to those reported in the literature as g C/cm^2, a surface area of 7.5 cm^2 was used for each sample, based on the approximate length and diameter of the twig segments analyzed. Fungal biomass ranged from 0 to 0.49 mg/cm^2 for birch and 0.13 to 0.38 mg/cm^2 for hemlock.

On both an areal and a mass basis, fungal biomass was in the range of values for oak and poplar veneers reported by Tank and Webster (1998), who found means of 0.37 mg/cm^2 and 0.05 mg/cm^2 in two streams. Crenshaw *et al*. (2002) found approximately 0.20 mg/cm^2 on oak veneers incubated on a high-elevation, open-canopied streambed, and this is similar to the mean of 0.22 mg/cm^2 in the present study. Although the surface area:volume ratios of commercially available wood substrates are typically higher than those of natural substrates used in wood decomposition studies (Spänhoff and Meyer 2004), the surface area:volume ratio of the twig bundles used here (around 20) more closely resembles that of a veneer than that of a stick with a diameter of a centimeter or more. A 1 cm-diameter stick that is 15 cm long has a surface area:volume ratio around 4. A veneer with dimensions of 2.5 cm by 15 cm by 0.1 cm (Tank and Webster 1998) has a surface area:volume ratio of 21.2, which is identical to many of the commercially prepared substrates reviewed by Spänhoff and Meyer (2004). In contrast, the veneers used by Gulis *et al*. (2004) had a thickness of only 0.5 mm, meaning that they had a surface area:volume of around 43. Fungal biomass measured on these veneers under ambient stream conditions averaged around 24.5 mg/g AFDM, which exceeds even the measurements made in the Agüera catchment (Díez *et al*. 2002). Stelzer *et al*. (2003) also used thin veneers (0.5-0.7 mm) and found fungal biomass ranging from near 0 to approximately 23 mg/g AFDM. Tank and Dodds (2003) found more fungal biomass on veneers than on *in situ* stream wood, but the veneers had been in streams for only 21 d in most cases, while the natural wood presumably had experienced a longer fungal colonization period.

The overall mean respiration rate on twigs was 21 μg O_2 g AFDM^{-1} h^{-1}. The mean respiration rate on sticks naturally occurring in a Coweeta stream was 1 μg O_2 g AFDM^{-1} h^{-1} (Tank *et al*. 1993), which is equivalent to the minimum rates measured in this study. The sticks were larger (1-3 cm in diameter) than mine and likely represented a variety of species and stages of decay. Average respiration rates on oak veneers (Gulis *et al*. 2004) and birch veneers (Stelzer *et al*. 1993) with ample surface area were similar to the highest respiration rates I measured on birch twigs. Minimum respiration rates on oak veneers and on birch and hemlock twigs were similar, but peak rates on veneers exceeded those on twigs by about 100 μg O_2 g AFDM^{-1} h^{-1}. This difference reflects the fact that over three years, the highest respiration rates on oak veneers occurred in the summer (Gulis *et al*. 2004), when I collected no data. Summer maxima and winter minima were evident in both microbial respiration and fungal biomass on oak veneers (Gulis *et al*. 2004). Fungal biomass and respiration rate on birch and hemlock twigs appeared to follow the same pattern, although my study covered only seven months (Figs. 1 and 2). The winter relative maximum in both parameters, one of three temporal peaks that occurred, coincided with an extended "January thaw" at Coweeta. Fungal biomass and breakdown rate of leaves in a first order stream in Nova Scotia also showed winter minima and summer maxima, but the same parameters measured on decaying wood (birch popsicle sticks) remained low throughout the year (Nikolcheva and Bärlocher 2005). In contrast,

fungal biomass on poplar veneers was higher in the winter than in the summer during two experiments conducted at Coweeta (Tank and Webster 1998).

Breakdown rates (0.402 y^{-1} for hemlock and 1.168 y^{-1} for birch) were higher than most of those published for sticks and dowels, but were lower than or similar to the breakdown rates of chips and veneers (as reviewed by Díez *et al*. 2002 and by Spänhoff and Meyer 2004; Spänhoff and Gessner 2007). Birch and hemlock twigs broke down faster than white pine sticks < 15 mm in diameter (0.102 y^{-1}) and tulip poplar sticks < 20 mm in diameter (0.226 y^{-1}) incubated in streams at Coweeta (Webster *et al*. 1999). They also broke down faster than red oak sticks 1-3 cm in diameter (0.107-0.281 y^{-1}, Webster and Golladay 1988) and red maple sticks 0.5-1.5 cm in diameter (0.113-0.157 y^{-1}, Eggert and Wallace 2003), all of which were incubated in Coweeta for up to 4.5 y. Birch and hemlock twigs broke down about as fast as tulip poplar sticks in Hertzler Branch, Coweeta, but faster than tulip poplar sticks in Cunningham Creek and white pine sticks in Hertzler Branch (Webster *et al*. 1999).

These differences probably resulted from the small diameters and numerous branches of birch and hemlock twigs used here, which provide abundant surface area and contain very little heartwood; from a high degree of physical fragmentation of the twigs; and from the twigs' short incubation time (seven months). On average, 17% of hemlock twig mass was lost between days 0 and 42, and only 10% was lost between days 42 and 210, a period four times as long. Birch twigs also experienced a large (14%) loss of mass by day 42, but, in contrast to hemlock, also lost a large proportion (17%) between the penultimate and last collections, a 28-day time span.

Although comparisons of microbial colonization and activity between birch and hemlock sticks showed that hemlock sticks provide a relatively poor food source and a relatively resistant substrate, the high surface area of fine twigs appears to make them accessible to microbes. While larger sticks and boles are exceptionally slow to break down (Harmon *et al*. 1986), small sticks support about as much microbial activity as thin veneers. This ability to host fungi and bacteria suggests that hemlock twigs are an important site of heterotrophic activity in summer, when leaves are absent.

Reach-Scale Dynamics

Short ammonium-N uptake lengths (< 100 m) indicated that through most of the year, ammonium travels only short distances before being taken up by biota. Uptake length and uptake velocity were both seasonal in character, suggesting that temporal variation was not due simply to variation in flow but was the result of biotic activity. A similar pattern has been previously documented in a stream at Coweeta and was attributed to changes in leaf abundance and heterotrophic activity associated with leaves (Tank *et al*. 2000). The difference in the relationship of leaves and uptake velocity between the two streams in the present study (*i.e.*, a significant relationship in Little Hurricane Branch, no clear relationship in Camprock Branch) (Fig. 8) is attributable largely to differences in the timing and quality of leaf inputs to the streams (Fig. 5), although neither stream experienced simultaneous peaks in uptake velocity and leaf standing crop (Fig. 8).

In both reaches of Little Hurricane Branch, leaf standing crops (Fig. 5b) and V_f (Fig. 7) increased from September to October. Between the October and November

collections, a slight decrease in leaves occurred in the reference reach compared to a larger drop in the addition reach. The reference reach experienced a slight increase in uptake velocity during this time, presumably the result of conditioning of the leaves, while uptake velocity in the addition reach decreased. Leaf standing crops did not change between November and January in the hemlock addition reach, suggesting that leaves there were strongly retained and were being heavily colonized by microbes. Microbial growth on leaves, along with a rise in temperature preceding the January sampling and a large standing crop of benthic organic matter, contributed to the high uptake velocity in this reach in January. The hemlock twigs in the addition reach may have substantially increased the surface area of organic matter available to microbes relative to the other reaches. From January to March, uptake velocity decreased with leaf standing crop in both reaches of Little Hurricane Branch.

Camprock Branch showed a different pattern. In October, the reference and treatment reaches had equivalent standing crops (Fig. 5b) but dissimilar uptake velocities (Fig. 7). Leaf standing crops were largest in both reaches in January, and both reaches probably experienced substantial inputs of late-falling oak leaves and blown-in leaves of various species. The stream reach is located between steep hillslopes, and sections of it have very little rhododendron cover, making it especially prone to intercepting fallen leaves. These senesced, unconditioned leaves are associated with a drop in uptake velocities in January. Other factors contributing to the decline include low temperatures preceding the January sampling (which would limit colonization of leaves) and increased background NH_4-N concentrations.

Peak uptake velocities were similar to or slightly lower than other measurements of ammonium uptake made at Coweeta in fall and summer (Table 3) using ^{15}N tracer injections. The differences are likely the result of differences in technique, since absolute enrichments are known to underestimate uptake relative to isotopic enrichments (Tank *et al*. 2000, Payn *et al*. 2005).

Table 3. Ammonium uptake velocity measured in the present study and others conducted in forested southern Appalachian streams.

Reference	Study Location	Time of Year	Method	V_f (mm/min)
Tank *et al*. 2000	Ball Creek, NC	Fall	^{15}N addition	16.2 – 44.4
Hall *et al*. 1998	Hugh White Creek, NC	Summer	^{15}N addition	2.4
Mulholland *et al*. 2000	Walker Branch, TN	Spring	^{15}N addition	6.0 – 8.4
Webster *et al*. 2000	WS53, NC	Year-round*	Enrichment	6.6
This study	Camprock Branch and	Sep.	Enrichment	0 – 9.79
	Little Hurricane	Oct.-Nov.		7.61 – 24.0
	Branch, NC	Jan.		5.36 – 19.5
		Mar.		3.11 – 7.79

*Mean of quarterly measurements made over 6 y.

Areal uptake in the birch treatment reach was particularly high in March (Fig. 9), when the background NH_4-N concentration was elevated in comparison to the other reaches (2.80 µg NH_4-N/L in the birch treatment reach vs. 1.48, 1.27, and 1.62 µg NH_4-N/L). Background concentration did not appear to be an important cause of the peak in the hemlock treatment reach in January (4.85 µg NH_4-N/L in the hemlock treatment reach

vs. 5.40, 4.70, and 4.61 μg NH_4-N/L in the other reaches). Seasonal changes in background concentration—high in September, low in October and November, and high in January and March—could be due to high autumnal demand drawing down the available ammonium. However, the highest uptake measured occurred in the hemlock treatment reach in January, when the reach had a high background concentration just like the other reaches, which all experienced much less uptake than the hemlock treatment reach.

CONCLUSIONS

Hemlock twigs provide a poorer-quality substrate for microbial colonization and growth than do birch twigs, and, as a result, birch twigs break down more quickly than hemlock twigs. The low quality of hemlock twigs may be offset by the potential for the sticks to be a relatively useable substrate, due to their high surface area, when leaves are scarce. Although hemlock twigs appear to be effective in retaining leaves, they do not affect organic matter accumulations enough to substantially affect heterotrophic uptake of ammonium. Ammonium uptake is strongly affected by the timing of leaf inputs as it relates to leaf quality, leaf conditioning, and stream temperature. Although hemlock death may subtly change patterns of organic matter accumulation and breakdown, the potentially important effects of hemlock death include changes in large wood inputs, changes in hydrologic regime, and increases in rhododendron cover.

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