NON-ADDITIVE EFFECTS OF MIXED-SPECIES LEAF-LITTER ON A BENTHIC STREAM COMMUNITY IN THE PACIFIC NORTHWEST

by

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ABSTRACT

Organic matter inputs from three riparian tree species (red alder, western redcedar, and western hemlock) were investigated for food resource value to stream macroinvertebrates and fungi. Leaf-litter species were tested both individually and in mixed-species combinations (7 treatments) using two sets of stream mesocosms. Within the initial 3 weeks, ergosterol content (an index of fungal biomass) showed little variation between litter types. Differentiation started around 6 weeks and progressed over the 12 week duration of the experiment to where fungal accrual in hemlock litter was significantly higher that that of alder or cedar. In one set of mesocosms, macroinvertebrate biomass was significantly higher in alder-containing treatments, particularly for the shredder-detritivore species, while chironomids such as *Chaetocladius* and *Heterotanytarsus* were significantly more abundant in conifer-only treatments. No increase in shredder abundance or biomass coincided with the increase in ergosterol content, even when hemlock litter attained a peak of 810 μg ergosterol/g detritus. Shredder-detritivores were the group most affected by mixed-leaf species treatments. Data from single-litter channels were used to generate predicted abundance and biomass of macroinvertebrates for mixed-litter channels. Comparisons of predicted versus observed values in mixed-litter treatments found significantly higher 'non-additive effects' in abundance and biomass for alder-cedar and in biomass for alder-hemlock treatments. These effects were due primarily to the shredders *Zapada* and *Lepidostoma*, which achieved a higher abundance and biomass than predicted. Individual *Zapada* were also larger in alder-cedar and alder-hemlock treatments. A potential mechanism for this non-additive effect may be associated with faster accrual of fungal biomass on the alder leaf-litter when in mixed alder-conifer litter combinations compared to single-litter treatments. Results differed between mesocosms, and in one set of mesocosms with lower overall macroinvertebrate densities, no distinct pattern was evident. These results suggest a fungal mediated improvement in resource quality when in mixed-litter combinations.

Key words: leaf-litter, mixed-species litter, detritus, non-additive effect, macroinvertebrate biomass, aquatic hyphomycetes, ergosterol, fungal biomass, shredder, *Zapada, Lepidostoma, Brillia retifinis*, community composition, stream

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DEDICATION

To the scientists and scholars, whose efforts I have endeavored to build upon, and those pioneers of the future whose discoveries are yet to be realized.

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INTRODUCTION

In detrital-based systems, such as temperate forests, leaf-litter type, quantity, and timing are important to the biotic community as a primary energy resource (see Seastedt and Crossley 1984, Graça 2001 for reviews). Having a variety of vegetation types within the forest canopy is common. This vegetation provides a mixture of predominantly seasonal allochthonous material to the system (Richardson 1992a, Fyles and Fyles 1993). When the detritus is present as a mixture of leaf-litter species, there is the potential for a modification of the detritivore community, conceivably through the more efficient use of the variety of resources that are available. These effects on the biota are not necessarily predictable based on findings from individual litter types (Blair *et al.* 1990, Kaneko and Salamanca 1999). There is the potential of non-additive effects on the faunal community that could be inhibitory or facilitative in nature, particularly for obligate detritivores. Exploring the effects of leaf mixtures on the detritivore community in aquatic systems can further advance understanding of the processes involved.

In headwater streams, detritivore biota have, in the past, been typically viewed as being more prominently influenced by environmental variables, such as temperature (Ward and Cummins 1979, Webster and Benfield 1986), rather than leaf-litter. This opinion has evolved to encompass availability and type of organic matter resources as prominent factors in the distribution of the stream biota. Detrital organic matter sources are important for the population dynamics (Richardson 1991, Wallace et al. 1999) and timing of life cycles (Petersen and Cummins 1974) for organisms that consume this resource. Different types of detritus not only provide different nutrient resources that affect detritivorous organisms' growth rates (Bärlocher and Kendrick 1973, Sweeney and Vannote 1986), but also provide different microhabitats depending on the physical shape of the litter (Richardson 1992b).

Non-additive effects resulting from mixing of leaf-litter species have been found in >50% of cases of terrestrial litter mixtures, where decomposer abundance was not predictable based on their responses in single-species litters (see Gartner and Cardon 2004 for review). Mixed leaf-litter studies, investigating differences in forest dwelling macroinvertebrate decomposer communities, showed that the effects in mixed-litter packs were not predictable based on those with a single-litter type (Blair *et al.* 1990). The mixing of litter species had created both more chemical variability and structural complexity than the single species types (Kaneko and Salamanca 1999).

Temperate headwater streams are similar to terrestrial forest systems in that the main source of detrital organic matter is predominantly from the seasonal leaf-litter from adjacent and overhanging riparian vegetation (Ross 1963). It has been suggested that leaf-litter is a substantial component (50 to 99%) of the energy flow in small streams through the leaf-litter breakdown processes (Bärlocher and Kendrick 1974). In general, stream research has focused on evaluating responses of the biota to one or more types of detritus individually (Irons *et al.* 1988, Gessner and Chauvet 1994, Friberg and Jacobsen 1999), and only rarely in combination (Sweeney and Vannote 1986, McArthur *et al.* 1994, Swan and Palmer 2004). The effects of riparian tree species composition are particularly relevant to stream systems as restoration efforts places a heavy emphasis on riparian vegetation as indicators of biological integrity (Landers 1997). Determining the effects of forest management practices that modify the riparian forest canopy, particularly the red alder (*Alnus rubra* Bong.) component, is important to the function of small headwater streams in high-gradient mountainous areas of the Pacific Northwest (Piccolo and Wipfli 2002, Hernandez *et al.* 2005).

The relationship between detritivorous invertebrates and leaf-litter types is often considered to be mediated through the associated microbial fauna that grows on the litter (see Cummins *et al.* 1989, Gessner 1999, and Graça 2001 for reviews). Colonization of the leaf-litter by microorganisms alters the leaf tissue by increasing protein content, improving palatability, and having an overall higher nutrient values for detritivores (reviewed by Suberkropp 1998a). Fungi are the predominant microbial component associated with this modification on the larger (>1 mm)

pieces of leaf-litter (Chamier *et al.* 1984, Suberkropp and Klug 1976, Findlay and Arsuffi 1989). On leaf tissue, fungal growth increases the nutrient value of the litter due, in part, to the increased fungal biomass (mycelium occupy up to 10-15% of the leaf tissue), but also by the excretion of enzymes that are able to digest leaf-litter components (pectin, xylan, and cellulose) and transform inedible plant material into simple compounds (reviewed by Graça 2001). Fungal growth can be dramatically affected by leaf-litter type where fast decay-rate leaf species typically attain a higher peak content and in a shorter time period than leaf species that have slower decay-rates (Cummins *et al.* 1989, Gessner and Chauvet 1994). Fungi also have the ability to obtain a significant proportion of their nutrients from the water flowing over the leaf surface (Suberkropp and Chauvet 1995). As mixtures of leaf-litter species would provide greater variety of organic compounds into the water column as leachates (McArthur and Richardson 2002), there is the potential that fungal growth will vary when present in litter mixtures. This is supported by evidence from terrestrial systems where higher than expected microbial respiration was apparent in spruce and pine needle litter mixtures compared to spruce alone (Chapman *et al.* 1988). This depicts fungi as a key component of detritivore use of leaf-litter types, and suggests that fungal dynamics may differ when mixtures of leaf-litters are present.

Stream detritivores exhibit clear responses to different leaf-litter species based on varying qualities. A common measure of leaf-litter quality is a detritivore's preference for leaf-litter types that enhance their growth rate (Ward and Cummins 1979, Sweeney 1993, Canhoto and Graça 1995). This preference is influenced in part by the degree of fungal colonization of the leaf (Suberkropp and Klug 1976, Bird and Kaushik 1985), the fungal species able to colonize the leaf type (Suberkropp and Klug 1976, Bärlocher 1982), as well as biochemical differences that may result from the same fungal species growing on different nutrient sources, such as different species of leaf-litter. Field studies of natural invertebrate populations have demonstrated that the variation in leaf-litter quality is reflected not only in the growth of detritivores, but their abundance as well (Otto 1974, Webster and Benfield 1986, Hernandez *et al.* 2005).Leaf-litter species quality cannot be directly inferred by their in-stream decay-rates. Conifer needles generally decay at lower rates (Sedell *et al.* 1975) and were thought to serve a limited role in detritivore nutrition compared to

deciduous litter species (Webster and Benfield 1986, Friberg and Jacobsen 1999). However, this was not the case for all conifer litter types or slow processing rate litters, and varies with the litter species examined. For instance, a slow decay-rate conifer species (western redcedar – *Thuja plicata* Donn ex D. Don) has been shown to have similar detritivore abundance as red alder, a fast decaying, deciduous species (Richardson *et al.* 2004).

Collectively, this supports the premise that alteration of the riparian forest species composition can affect shredder species' growth rates (Bärlocher and Kendrick 1973, Sweeney and Vannote 1986, Richardson 1991, Friberg and Jacobsen 1999), and has the potential to affect their abundance and diversity (Cummins *et al.* 1989, Piccolo and Wipfli 2002).

Experimental objectives

In this study, we present data on the effects of leaf-litter quality and mixtures of leaf-litter on stream communities, including fungi and macroinvertebrates. Forested, headwater streams of the Pacific Northwest are well suited for this experiment as they are typical of these detrital-based systems with most of the energy flow coming from leaf-litter detritus that is a mixture of deciduous and conifer riparian vegetation (Richardson 1992a). Alder litter has been extensively tested in stream systems. However, for the two conifer species, little published information is available on the fungal dynamics on western redcedar (Summerbell and Cannings 1981) and none for western hemlock (*Tsuga heterophylla* Sargent).

In the framework of this stream system we ask the following questions: 1) What is the rate of fungal growth and maximum biomass attained on the three types of riparian tree species leaf-litter (alder, cedar and hemlock)?; 2) Is detritivore macroinvertebrate use of leaf-litter related to fungal biomass accrual on the leaf-litter?; 3) How is the detritivore macroinvertebrate community (composition, diversity, and biomass) affected by the three different leaf-litter types and combinations beyond what is predicted by fungal biomass accrual?; and 4) Is the detritivore macroinvertebrate community response to combinations of leaf-litter ($\geq$ 2 leaf species) predictable based on the response to the single leaf-litter types?

MATERIALS AND METHODS

Study site

The study was conducted in two sets of experimental stream channels adjacent to Mayfly Creek (49°16'N, 122°34'W) in the University of British Columbia's Malcolm Knapp Research Forest in Maple Ridge, BC. The research forest is located in the Coast Mountain Range approximately 60 km east of Vancouver, British Columbia, Canada. The predominant vegetation in the watershed was second growth conifer forests of western hemlock, western redcedar, and Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco], with deciduous species such as red alder, vine maple (*Acer circinatum* Pursh), and salmonberry (*Rubus spectabilis* Pursh) in the riparian areas.

The two sets of artificial stream channels, or stream mesocosms, were spaced >1 km apart along Mayfly Creek. Mayfly Creek is a fishless, second-order, oligotrophic headwater stream located in the Coastal Western Hemlock biogeoclimatic zone. The source creek adjacent to the mesocosms had a boulder, gravel, cobble and sand substrate at the lower site (Lower Mayfly Creek - LMC) and a slower flow, gravel and sand substrate at the upper site (Upper Mayfly Creek - UMC). LMC is at an altitude of 315 m, with an average bankfull width of 3 m and average slope of 0.08 m/m. UMC is about 100 m higher in altitude with a lower slope. Temperature ranges during the study were from 5.5 to15.1°C. Both sets of mesocosms had similar temperatures. In nearby creeks the water had average concentrations of phosphate (PO_4^+) of 10 μg/l, NO_3^- of 230 μg/l, and Ca^{2+} of 1.5 mg/l, pH of 6.8, and conductivity of 200 μS/s, which vary with season and discharge (Feller and Kimmins 1979, Kiffney *et al.* 2000). This area received a typical northern maritime climate of cool, wet winters and warm, dry summers. As a result discharge in Mayfly Creek is high and variable during winter while being lower and more stable during the summer and early autumn months.

Experiment design

The study was conducted over a 12 week period from August to October 2000. Three types of tree leaf-litter – red alder, western hemlock, and western redcedar – were used in the experiment. These tree species were chosen due to their prominence in the watershed, as well as their difference in physical and chemical characteristics. The litter was tested individually and in combination creating seven treatments: alder (A), cedar (C), hemlock (H), alder and cedar (AC), alder and hemlock (AH), cedar and hemlock (CH), and alder, cedar and hemlock (ACH). The two sets of mesocosms were used in a complete block design (2 blocks) to obtain 4 replicates of each treatment. The use of stream mesocosms allowed for the control of within and between-stream variation that depended on the local stream flow, substrate and depth characteristics (Corkum 1989), as well as biogeomorphic characteristics, temperature, shading, stream bed characteristics, other sources of organic matter, and woody debris.

Water from Mayfly Creek was diverted into the mesocosms (a total of 14 channels in each set). In LMC each channel was 14 m long and about 25 cm wide, with a slope of approximately 0.03 m/m (see Richardson 1991 for additional detail). UMC was 7.5 m long and 20 cm wide in an area shaded by hemlock adjacent the source stream (see Melody and Richardson 2004 for additional detail). Due to the differences in creek gradient and water source availability, the average flow in the channels at LMC was higher than in UMC. Flow variation over the duration of the study was 0.25 to 0.35 L/s per channel for UMC and 1.1 to 1.7 L/s per channel for LMC due to the changes in water level in the source creek. Due to the overhang of trees at the UMC set of channels which created 85% shading, fiberglass screens were placed overtop of the mesocosm and cleared off periodically of any hemlock needle debris. LMC channels were in a large clearing with full sun exposure where little debris could fall into the channels.

To ensure removal of all organic matter, channels were washed and a substrate of rounded glacial till collected from a nearby quarry was placed in both sets of mesocosms. Sizes varied from 0.5 to 4 cm diameter, with a small amount of sand added (45% of 3 to 4 cm, 30% of 1 to 2 cm, 20% of 0.5 cm, and 5% sand). The substrate was placed in the mesocosms to a depth of 10 cm in a

uniform riffle habitat. Irregularities in the gravel surface retained detritus and provided habitat for invertebrates (Richardson and Neill 1991). Water was diverted from the creek and passed through settling tanks to reduce suspended mineral sediment inputs and allow for immigration of invertebrates and import of fine organic matter from the creek, before being split into several pipes to supply the input for each experimental channel. A mesh screen was also used at the water surface of the settling tank to reduce the flux of course particular organic matter (CPOM) into the experimental streams. This screen did not block the flow into the mesocosm and invertebrates were able to pass freely.

The mesocosms were inoculated with stream biota by allowing stream water to pass through the channels for one week before starting the experiment. In order to ensure the full community of organisms were present in the channels, an equivalent area of Surber and kicknet samples were also taken from Mayfly Creek and used to inoculate the channels. Community composition and abundance of macroinvertebrates in the LMC mesocosm and Mayfly Creek were previously shown to be similar (Richardson 1991).

Leaf-litter

Recently abscised leaf-litter was collected from locally available red alder, western hemlock, and western redcedar trees over the course of the preceding, and concurrent fall season. Litter was collected by laying down fiberglass mesh on the ground to minimize contact with soil biota. Collected litter was air dried and weighed in the laboratory. Time of initial fungal colonization of the litter was potentially an artifact of the drying process of the litter. Drying breaks down the leaf cell structure and promotes access to microbes (Gessner 1999). However, drying leaf-litter was conducted in order to standardize the total amount of organic matter added to each experimental channel.

Litter was added to the channels at a rate of 2.5 g dry weight/m^2/day. This rate was chosen as an approximation of a moderate fall input rate based on litter input rate data collected for Mayfly Creek in the late 1980s (Richardson 1992a). For ease of design, additions were done every 10 to 11

days from August to September for a total of 6 litter additions. Litter additions ceased after the end of September due to accumulation of the added organic matter in the channels. Emigration nets were placed at the downstream end of the channels when the litter was added, and litter that passed through the channels after 24 hours was returned to the top of the channel to minimize loss of organic matter inputs and increase consistency between treatments. Benthic invertebrate and fungal pack sampling continued until the end of October.

Fungal biomass assay

In order to distinguish between the different times of litter additions, at the initial litter addition five leaf-litter packs of 2.0g and one of 5.0g were added to determine fungal biomass and decomposition rates, respectively. Leaf-litter pack material consisted of a soft, pliable 1 mm mesh netting. This size was used in order to contain the hemlock needles and allow macroinvertebrates to enter the leaf pack. Shredder species were found within the packs but the mesh excluded the larger individuals. Generally, invertebrate shredder species' exclusion has been carried out with fine mesh bags of 0.5 mm or smaller (Bärlocher and Graça 2002), while invertebrates are able to colonize in a mesh size of 1.5 mm and larger (Stout and Coburn 1989).

Packs were removed from the stream, placed in opaque jars, and transported in a cooler to the laboratory every 3 weeks during the course of the experiment with an additional collection on day 11. On the same day, packs of leaf-litter were rinsed with water through a 1 mm and 63 μm sieve, invertebrates removed, litter placed in opaque film canisters, and frozen (-20°C) to minimize degradation of the fungal material. Fungal biomass estimates were determined by quantifying the amount of ergosterol present in the leaf-litter - a cell wall sterol largely restricted to eumycotic fungi (Newell 1992). Freeze-dried leaves were ground and sub-sampled (50 mg). Mixed-litter species packs were separated into their litter types based on physical characteristics before grinding into fine particles and sub-sampled. Lipids were extracted with alkaline methanol and the extracts cleaned by solid-phase extraction with a C18 reverse-phase extraction column (Varian Bond Elut C18, 3 mL column with 500 mg sorbent). The elutes were then separated and quantified by high

performance liquid chromatography (HPLC) –Waters 600E system controller with Waters 700 Satellite WISP. The ergosterol fraction was detected at ultra-violet (UV) absorbance of 282 nm after passing through a Lichrospher RP18 column (25 cm by 4.6 mm). The samples were run with 100% methanol at a flow rate of 1.5 mL / min. The procedure was based on a protocol developed by Gessner and Schmitt (1996) with some modifications with the extraction procedure and extraction column (Dr. S. Mansfield, pers. comm. - see Appendix for details).

Trial double extractions were conducted on the litter to ensure the duration of the extraction procedure with the Soxhlet extraction resulted in the removal of all the detectable ergosterol from the litter. In order to account for any ergosterol lost in the processing procedure, samples spiked with different amounts of pure ergosterol underwent the same procedure of extraction as controls. No conversion factors were used for ergosterol to fungal biomass as these have not been determined for the Pacific Coastal areas, and different fungal species assemblages can have variable amounts of ergosterol content (Gessner and Chauvet 1993).

Initial tests for ergosterol on the three species of leaf-litter demonstrated that alder contained minute amounts of fungal colonization at the beginning of the experiment, whereas cedar and hemlock did not contain any detectable amounts. These initial tests also indicated that the conifer species had no other chemical compounds that would interfere with the detection of ergosterol under these conditions on the HPLC at 282 nm UV absorbance.

Ergosterol levels were determined for all single-species and one mixed-species (ACH) treatment. A complete set of data for the ACH treatment was not possible as a precipitate developed in solution clogging the HPLC. As a result, only part of the data set was available for the ACH treatment where $n = 4$ for day 24, $n = 2$ for days 46 and 69, and $n = 1$ for day 87. Statistical tests including ACH ergosterol values were performed only on day 24 data sets.

Macroinvertebrate communities

Benthic samples were taken from the channels at six week intervals – day 42 and day 84. Samples were collected to a depth of 10 cm from the surface with a mini-Surber sampler with a sampling area of 289 cm^2 and a 300 μm net mesh. Both sets of stream channels had 6.1% of their bed area removed for each stream channel on each date sampled. Samples were collected from the channels in a stratified random design. Benthic samples were preserved in buffered formalin in the field for storage until processing.

In the laboratory, two samples per stream channel per date were randomly selected for processing. The samples were washed through a series of four sieves (4 mm, 1 mm, 500 μm and 63 μm) and Organic debris was separated from invertebrates with a dissecting microscope at 6x magnification. Only the invertebrates retained in the 500 μm or greater sieve sizes were identified. Aquatic insects were identified to genus level and their lengths measured from the top of the head to the tip of the abdomen. Measurements were taken of each individual and rounded to the nearest 0.5 mm under the dissecting microscope for each sample. Biomass estimates for all taxa were based on published length-mass conversions (Benke *et al.* 1999). Identification of common chironomid genera were confirmed by Dr. C. Gjerløv.

Statistical analyses

The study data were analyzed with analysis of variance (ANOVA, SPSS 12.0) with a complete block design using two sets of mesocosms (LMC and UMC) on Mayfly Creek. Each channel was a replicate providing a total of 4 replicates for each treatment. When the model was significant based on $\alpha \leq 0.05$, least significant differences (LSD) were used for *a posteriori*, pairwise comparisons. Normality of residuals from the ANOVA were tested with Shapiro-Wilk and Kolmogorov-Smirnov tests for normality.

An ANOVA was used to test for differences in percent of litter remaining with block, litter type (A, C, H), and number of litter types in the pack as the main effects. For the macroinvertebrate

data (biomass or abundances), an ANOVA was also used to test for differences among treatments with block and treatment as the main effects. The data presented focused on LMC channels and day 84 due to the larger (> 2x) invertebrate abundances present on average and an associated larger shredder-detritivore population. An unpaired t-test was performed on the abundance of some invertebrate genera where treatments were grouped into 2 categories: those with alder (A, AC, AH, and ACH) and those without alder in the treatment (C, H, and CH). An ANOVA was used to test for differences in ergosterol content with day, treatment, and block as fixed effects. With this approach the residuals were normally distributed with a mean of zero. A simple regression was used to test for a relation between fungal and insect biomass. Both UMC and LMC data were included in the analysis.

Principal component analysis (PCA) was employed for a visual assessment of the data (SYSTAT 10.2) for day 84. No transformations were performed and the data was based on the ten most abundant aquatic insects and two additional genera that were in the top ten for biomass, i.e., *Wormaldia* and *Paraleptophlebia*, for a total of 12 genera. This minimized the presence of zeros in the matrix. The analysis was performed on a square symmetric matrix of pure sums of squares and cross products. The PCA All 12 genera had at least one component loading of greater than ± 0.5 in at least one of the PC axes. Only PCs with eigenvalues over 1 were considered which resulted in 3 PCs. Species listed in the principal component scatter plot were those with component loadings of greater than the threshold of ± 0.3.

For mixed-litter treatments, an ANOVA was used to test the hypothesis that the actual detritivore abundance for leaf-litter species in mixed-litter treatments was different from that predicted by the average of the detritivore abundance (DAb) estimated for each of the component species individually (H_o: $DAb_{mixed} - [(\Sigma DAb_i)/n] = 0$, where i = single leaf-litter species present in the mixed-litter species treatment and n = the number of single leaf-litters included). If comparisons between the actual observed values and predicted values (actual - predicted) differed significantly from 0 based on a t-test, this indicated non-additive effects. If the abundance in the mixed-litter treatments were more than a numerical average predicted by the single-litter treatments, this

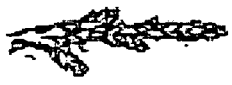
suggested facilitation; while if the mixed-litter treatments were less than that of the average predicted, this suggested inhibition (design from: Jonsson and Malmqvist 2000, Soluk 1993). If the findings from the mixed-litter treatments could be predicted by the average of the appropriate single-litter treatments, the effects were said to be 'additive'. If the dynamics of the mixed-litter types were not predictable relative to the single-litter treatments, the effect was considered 'non-additive'. Data from single-litter species treatments were combined to calculate predicted abundance and biomass of aquatic insects as a means of determining differences between mixed-litter versus single-litter treatments for day 84 data. Graphical display of mixed-species treatments were based on residuals of observed minus predicted values. Aquatic insects were examined on an abundance or biomass / unit area. Shredder insect species were further examined on a per gram organic matter (OM) basis (abundance or biomass / g OM).

RESULTS

i. Leaf-litter quality

The three leaf-litter types used in this study varied in nutrient content, chemical composition, physical shape, and susceptibility to microbial colonization (Table 1). The difference between ergosterol content of the litter types accentuates the qualitative dissimilarity between them. Alder litter had approximately double the nitrogen (N) content of the two conifer leaf-litters. This did not equate to higher maximum ergosterol content as hemlock had over twice the maximum value found for alder in both single and mixed-litter packs. The peak value for cedar was 32% higher than alder, but only in single-litter packs.

TABLE 1: Comparisons of the differences in leaf-litter characteristics. Values are means (±1SE) and maximum ergosterol is the peak value reached over time per g dry matter (DM).

Physical Characteristics	% Mass Remaining[1] single-litter packs on day 87	Maximum Ergosterol (mg / g DM)		Initial Nitrogen[4] (%)	Initial Lignin[4] (per g DM)
		Single-litter packs[2]	Mixed-litter packs[3]		
alder 	43.7 (2.2)	0.30	0.48	2.33 (0.02)	119.5 (1.9)
cedar 	73.0 (0.6)	0.44	0.42	1.07 (0.02)	100.5 (2.0)
hemlock 	62.6 (0.2)	0.81	1.01	1.30 (0.01)	91.1 (1.1)

[1]n = 4, [2]n = 2, [3]n = 1, [4]Richardson *et al.* 2004

ii. Leaf breakdown

The percent of the original litter remaining after 87 days differed significantly between the three litter types (ANOVA: $P<0.001$, model $r^2 = 0.84$). As originally predicted, alder had the highest rate of loss. The three litter types decayed at different rates based on LSD regardless of litter combinations (Figure 1). There was no significant interaction or block effect. Furthermore, no difference in species-specific amounts remaining was evident when the litter types were present in mixed or single-litter treatments.

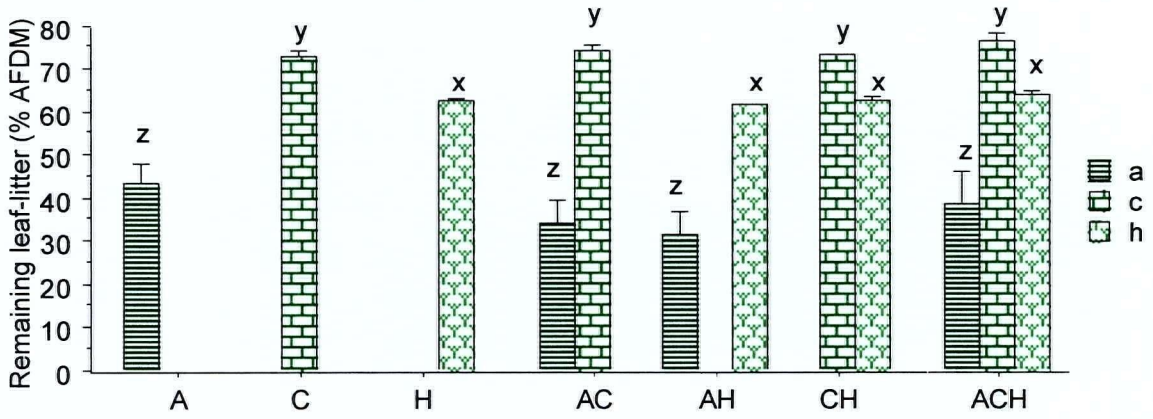


FIGURE 1: Comparison of the mean percentage of the original leaf-litter remaining (+1 SE) as ash free dry matter (AFDM) in leaf packs at day 87 for all 7 treatments, all with n = 4 (channels as replicates). The treatments are divided into their respective components for comparison purposes – alder (a), cedar (c), and hemlock (h). Bars with the same letter denote no significant difference from each other based on post hoc LSD.

iii. Fungal biomass

The extraction procedure was shown to effectively extract all detectable ergosterol from the litter. During leaf-litter decomposition in the stream, ergosterol content increased significantly at a moderate rate with peak values for the single-litter species being reached at different times. The ergosterol content on single-litter types differed significantly between treatments, between the two mesocosms, and over time (ANOVA: treatment $P < 0.001$, block $P = 0.001$, time $P = 0.002$, model $r^2 = 0.61$). Between the 2 mesocosms, differences in the rate of ergosterol accrual over time (block*time P=0.04) and in the peak concentrations were found for all three litter types (Figure 2). The two conifer species reached higher peak ergosterol levels in UMC compared to LMC channels, where on average LMC was 50% lower for hemlock and 45% lower for cedar. The timing of peak maxima was similar for both sets of channels with alder reaching a peak at 6.5 and cedar at 10 weeks. In alder, ergosterol content increased much faster in UMC channels, but reached a plateau 17% lower than the peak reached at LMC. In the LMC channels, cedar and alder leaf-litter decreased in ergosterol content after their peak biomass at a similar rate as their rate of increase. This symmetry was not evident in the UMC channels. Hemlock had the highest degree of variation among samples and a significantly higher ergosterol content compared to the other leaf-litter species (LSD: $P < 0.001$). Furthermore, hemlock ergosterol levels did not reach a defined peak but continuously increased over the duration of the experiment. The greatest rate of increase in ergosterol for hemlock was within the initial 6.5 weeks in both sets of channels.

The ergosterol content per gram of leaf tissue appeared to be variable when present in combinations of leaf-litter. On average, alder had a trend of containing higher ergosterol content in mixed-litter combinations but fluctuated though time. In contrast, cedar had a slight decrease in the mixed-litter packs, and hemlock showed no difference (Figure 3). The maximum ergosterol content attained per g of leaf tissue was higher when present in mixed-litter packs compared to single species packs for alder and hemlock (Table 1). However, the degree of increase in peak ergosterol content when present in mixed-litter combinations needs to be viewed cautiously as the high values for mixed-litter combinations on day 87 were based on single samples.

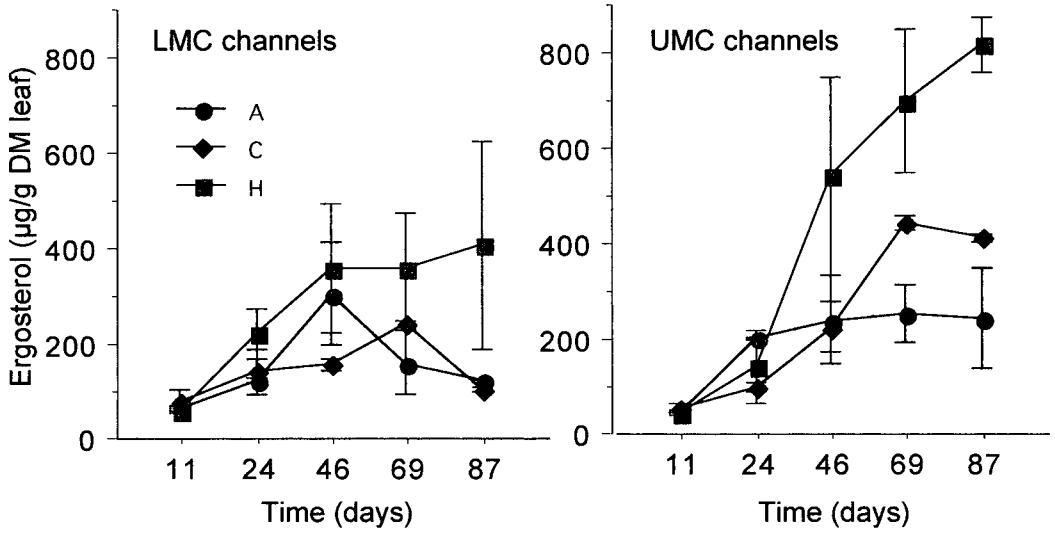


FIGURE 2: Ergosterol content associated with decomposing single species leaf-litter treatments of alder (●), cedar (♦), and hemlock (■) over 12 weeks. The mean in μg/g of dry mass of leaf material (±1SE) where n = 2 channels each for LMC and UMC.

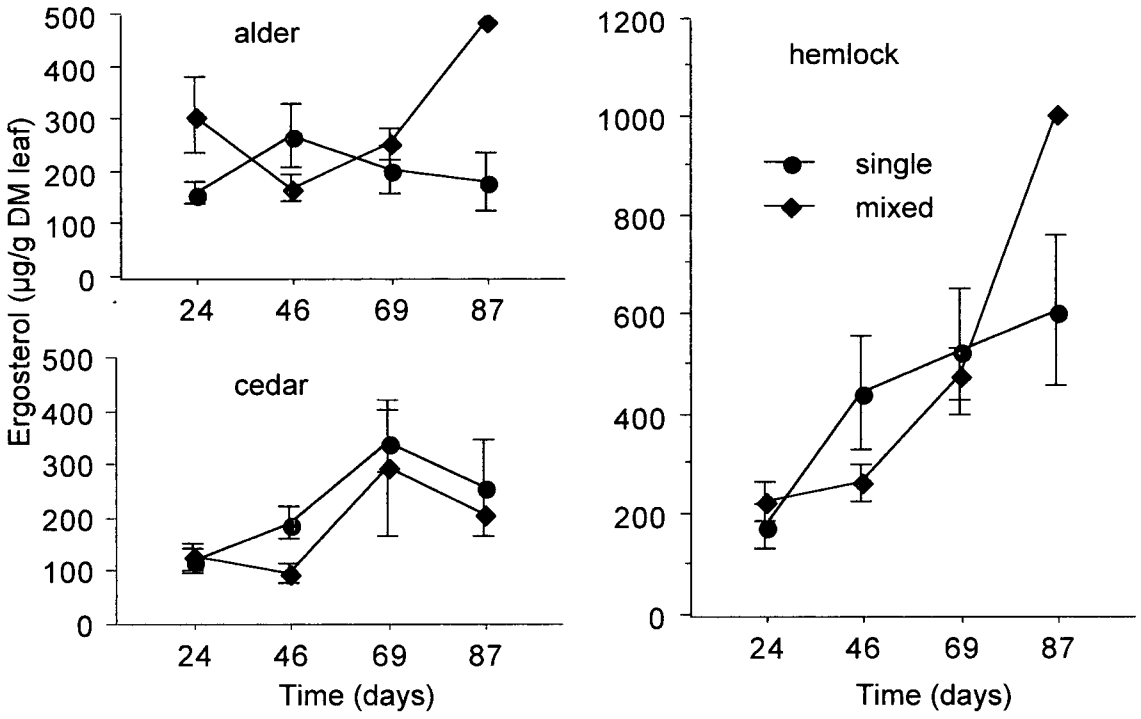


FIGURE 3: Ergosterol content for the 3 leaf-litter types in single (●) and mixed-litter packs of all 3 leaf-litter types (♦) separated into the alder, cedar, and hemlock litter types over 12 weeks averaged for both sets of mesocosms. The mean in µg/g DM leaf material (±1SE) where n = 4, 2, 2, and 1 for the respective dates for each of the leaf-litter types.

Overall, the main difference between single and mixed-litter fungal ergosterol content was the anomalous accrual pattern in alder litter. This difference was most obvious in LMC where the ergosterol content of alder reached a peak earlier and displayed a higher overall ergosterol concentration (400 μg/g DM at day 24) in the mixed-litter packs compared to the single-litter treatment (300 μg/g DM at day 46). For cedar in mixed-litter packs, timing of peak amounts and rates of change were similar in both sets of channels, while hemlock had the same timing of peak amounts but had a delayed rate of increase in UMC for the mixed-litter packs through time. After day 24, the ergosterol content of alder in mixed-litter packs decreased by half before increasing again by day 69.

Closer examination of the ergosterol levels at day 24 showed that mixed-litter combinations had significantly more ergosterol than the single-litter types (ANOVA: single vs. mixed-litter P = 0.036, litter type P = 0.020, block P = 0.036) where alder was significantly different from cedar (LSD: P = 0.007) and hemlock (LSD: P = 0.039). There were no significant interaction terms. At day 24, alder had 68% greater ergosterol content when present in a mixed-litter combination than as a single-litter species treatment for the LMC channels, but this difference was not evident in the UMC channels for this date (Figure 4).

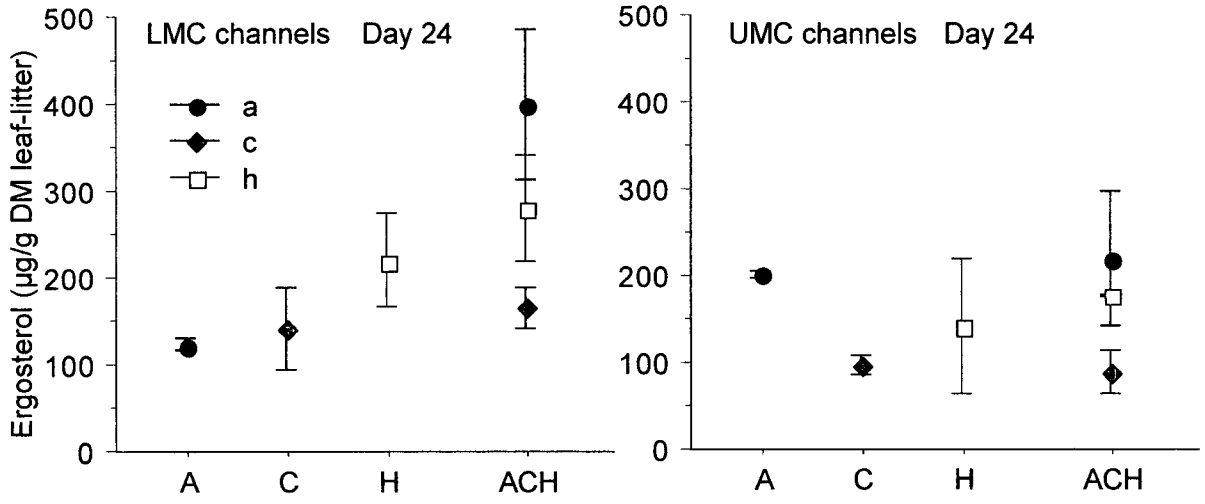


FIGURE 4: Ergosterol content in the single leaf species alder (A), cedar (C), and hemlock (H) and mixed-litter combination (ACH) treatments. The mixed-litter treatment was divided into its respective litter types – alder (●), cedar (◆), and hemlock (■). The mean in μg/g DM leaf material (±1SE) where n = 2 channels each for UMC and LMC.

iv. Invertebrate communities

Statistical analyses were conducted on the second invertebrate sampling date (day 84) only because there was a complete data set for all treatments and significantly greater abundance of insects on this date. Overall abundance in LMC was 2.5 to 3 fold higher and biomass was 2.5 to 5 fold greater on day 84 than those of the first sampling date (day 42) ($P < 0.001$). Similar findings were evident for UMC. The large increase in biomass was due to a species shift from being predominantly Chironomidae, to representation by a greater variety of aquatic insect families. Collector-gatherers were consistently the most abundant species on both sampling dates, however by day 84, the average biomass of shredder-detritivores was similar to collector-gatherers even though shredder-detritivores were not as numerous.

a. Abundance and biomass

Overall, there was no significant difference in macroinvertebrate response when abundance, biomass, or genera richness were examined on the different treatments, numbers of litter species in combination, or presence of alder in a treatment. The block effect was consistently significant with results specific to the different sets of mesocosms. Macroinvertebrate abundance and biomass were higher (> 2×) in LMC compared to UMC mesocosms ($P < 0.001$). LMC also had a correspondingly larger shredder-detritivore population with an average of 1643 individuals/m^2 while UMC channels had 692 individuals/m^2. The abundance of the Orders Plecoptera, Trichoptera, and Diptera all showed significant block (mesocosm) ($P \leq 0.001$) and biomass ($P \leq 0.02$) effects, while Ephemeroptera only showed a significant block effect for biomass ($P = 0.001$).

Responses to treatments were evident in LMC mesocosm. LMC had an overall trend of higher aquatic insect biomass for the AC and AH in mixed-litter combinations, while the CH mixed-litter combination was the lowest (Figure 5). In contrast, UMC had a greater abundance and biomass in the alder-only treatment.

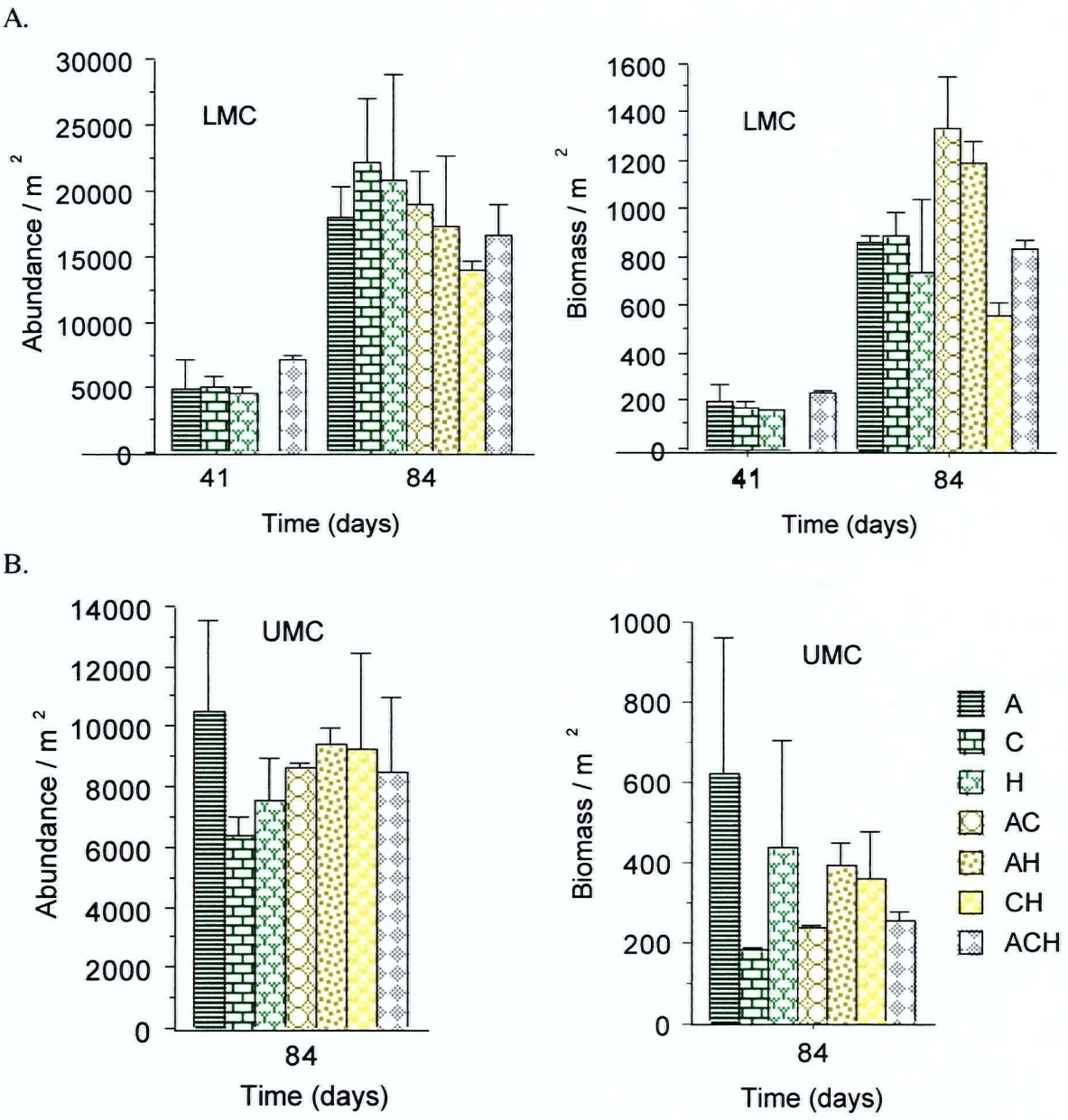


FIGURE 5: Comparison of overall benthic macroinvertebrate abundance and biomass in the different treatments for a) LMC and b) UMC. Mixed-litter treatment samples were not processed for mixed-litter treatments with 2 litter types on day 41 in LMC. The means (+1SE) are shown for two sampling dates collected with a miniature Surber sampler removing 578 cm^2 of benthos, where n = 2 channels. Abundance includes all macroinvertebrates, while biomass only includes aquatic insects.

b. Diversity indices

Numbers of macroinvertebrate genera did not differ significantly between treatments, but did double in number between sampling dates (day 41 and day 84 where $P < 0.001$) and displayed a significant block effect ($P < 0.001$). On average, the number of macroinvertebrate genera was 43 in LMC and 28 in UMC for day 84. When the effect of the rare genera are minimized through the use of Simpson's diversity index (1/D), the alder-only treatment demonstrated a significant effect when both mesocosms were included (ANOVA: treatment $P = 0.003$, block $P < 0.001$, model $r^2 = 0.69$). Alder had a significantly higher diversity of aquatic insects than the other treatments in either mesocosm (Figure 6).

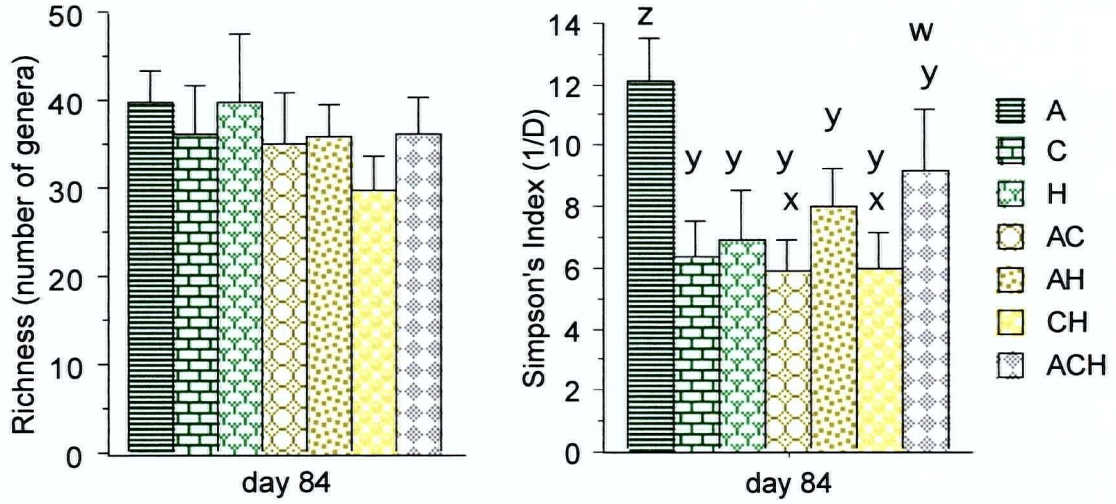
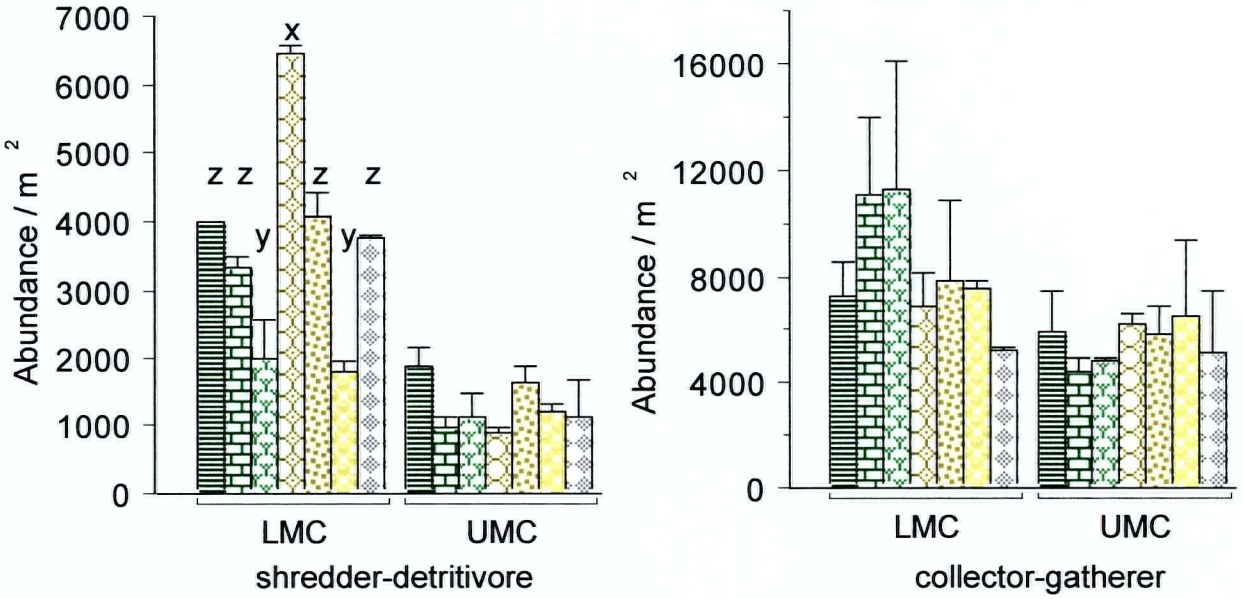


FIGURE 6: Comparison of macroinvertebrate diversity (number of genera and Simpson's index) for day 84 with both mesocosms included. The bars represent mean (+1SE) where n = 4 channels. Simpson's index showed that differences in diversity of the more abundant genera exist where bars with different letters were significantly different from each other based on LSD.

c. Functional feeding groups

When functional feeding groups (FFG) in both mesocosms for day 84 were examined the shredder-detritivores were significantly different in both abundance and biomass (ANOVA: treatment, block, and treatment*block were all $P < 0.001$ for abundance, model $r^2 = 0.94$ and $P \leq 0.001$ for biomass, model $r^2 = 0.83$). In LMC, the AC mixed-litter combination for abundance and biomass separated out from the other treatments except the mixed-litter AH combination (LSD: $P < 0.001$, $P < 0.007$, respectively) (Figure 7). Other FFG did not differ significantly between treatments. However, predators, scrapers, and shredder-herbivores were all significantly different between mesocosms (ANOVA: block $P < 0.001$).

A.



B.

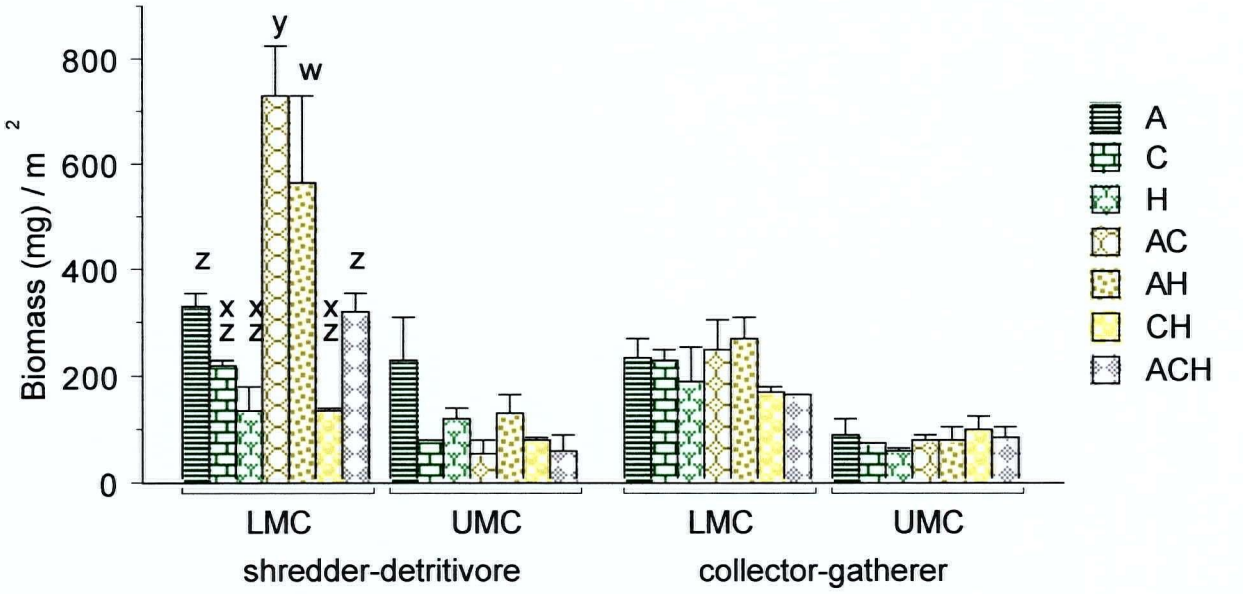


FIGURE 7: Comparison of the means (+1SE) for shredder-detritivore and collector-gatherer functional feeding group abundance and biomass from day 84 where n = 2 channels. Both UMC and LMC are shown separately. Part A: Shredder-detritivore abundance in LMC differed between treatments where bars with different letters were significantly different from each other based on LSD. Part B: Shredder- detritivore biomass differed between treatments where LSD showed that the bar with y is significant to those with z and the bar with w is significant to those with x.

The shredder-detritivore FFG abundance and biomass per unit organic matter differed significantly in LMC, but only between treatments with or without alder (t-test abundance P = 0.02, biomass P = 0.006). The shredders were higher in abundance and more than double the biomass in the alder-containing treatments compared to the treatments without alder (Figure 8).

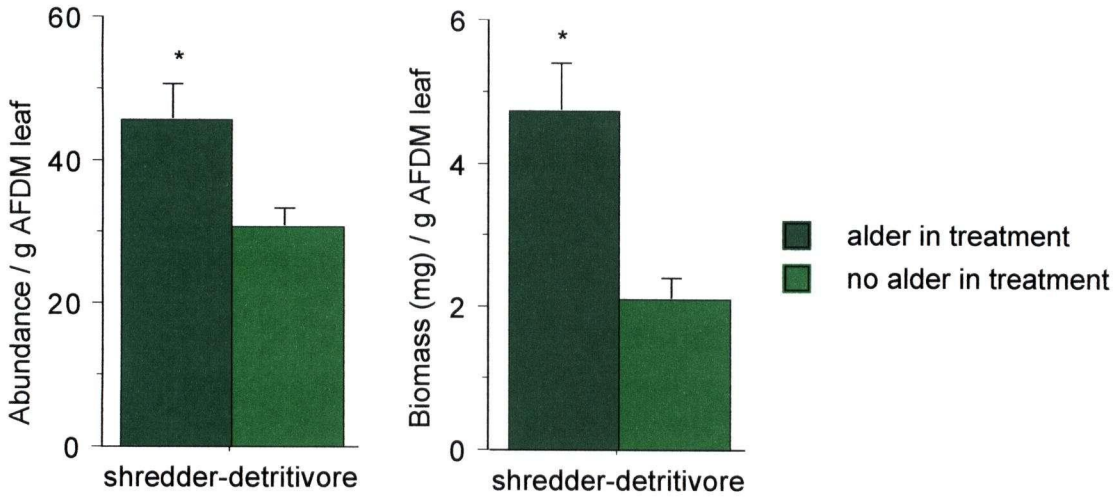


FIGURE 8: Comparison of the means (+1SE) for shredder-detritivore abundance and biomass per unit AFDM for LMC.

d. Common species

Changes in community composition occurred through time, with less variation between treatments evident on day 41 than on day 84. The abundance and biomass response of particular species varied among treatments. The community on day 41 was dominated by Chironomidae collectors and some shredder species (mostly *Brillia retifinis* with a few early instar *Zapada* and *Lepidostoma)*. The composition of this community displayed a trend towards higher abundance and biomass in the ACH treatments, but it was not significant. The community on day 84 had a prominent shredder component, with *Zapada* [Plecoptera: Nemouridae – primarily *Zapada cinctipes* (Banks) with some *Zapada haysi* (Ricker)] and *Lepidostoma* [Trichoptera: Lepidostomatidae – primarily *Lepidostoma roafi* (Milne) with some *L. unicolor* (Banks) and *L. cascadense* (Milne)] comprising the majority of the individuals (Figure 9).

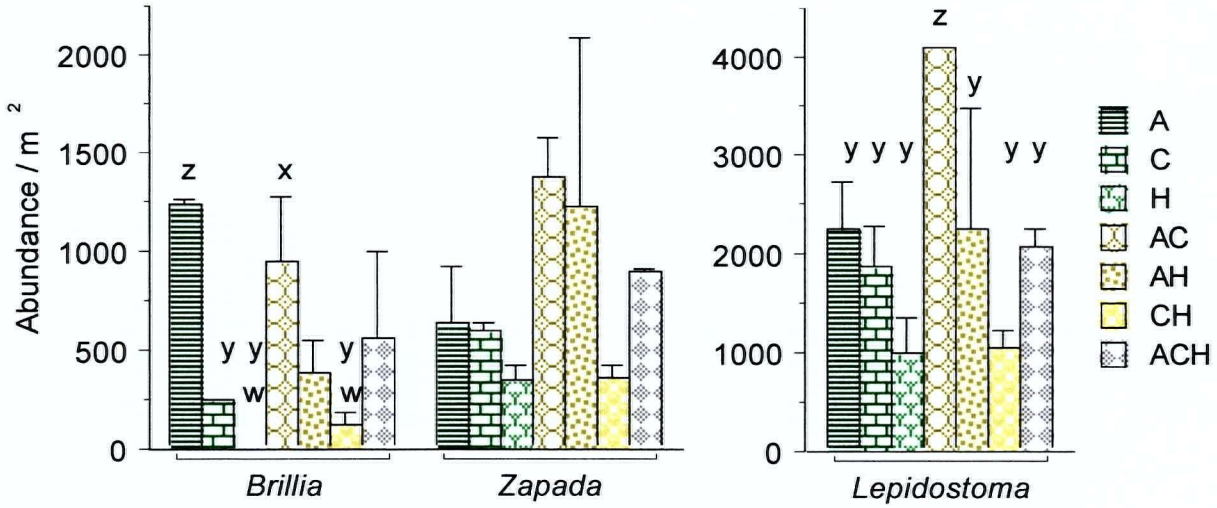


FIGURE 9: Comparison of the means (+1SE) for different shredder species abundance for LMC. *Brillia* abundance was significant where z is significant to y and x is significant to w based on LSD. *Lepidostoma* abundance was significant where the AC treatment (z) is significantly different from the others (y).

On day 41, *Brillia retifinis* was the predominant shredder species on all three litter species. However, by day 84, there was a significant effect of treatments on *Brillia* with a notable absence in the hemlock treatment (0 individuals) for UMC & LMC, even though fungal biomass was still increasing. In LMC, the LSD showed that the abundance of *Brillia* on A was significantly different from C, H, AH, and CH, while AC treatment was significantly different from H and CH. The results for biomass were similar to those found for abundance. *Brillia* abundance remained consistent on the cedar litter over time, but increased 2-fold on the alder treatment over the 43 days between sampling dates. On day 84, *Brillia* abundance was significantly higher in alder-containing treatments compared to conifer-only treatments (Table 2 & Figure 9). In LMC, *Brillia* were numerically 5-fold greater in the alder vs. conifer treatments. This significance was consistent when the data were examined on a per unit organic matter basis as well (Figure 10).

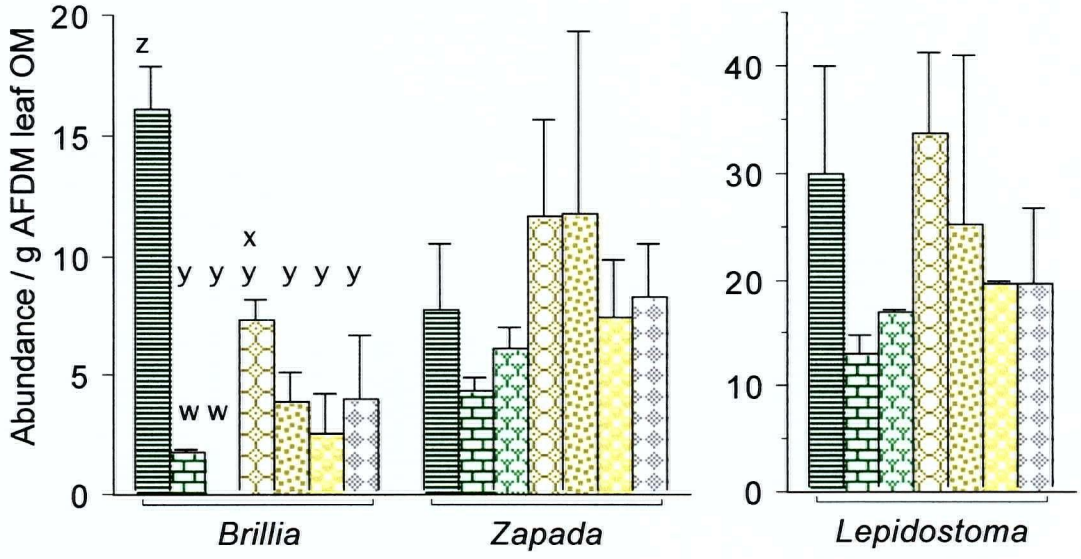
Trichoptera shredders were significantly more abundant in AC treatments in LMC compared to the other treatments (ANOVA: treatment $P = 0.03$, block $P < 0.001$, treatment*block $P = 0.01$). *Lepidostoma* was the most abundant of these shredders with a 2.9× greater biomass (mg/m^2) in LMC for alder containing treatments than in the conifer-only treatments (Figure 9), and 2.5× greater biomass (mg/g DM) when the amount of organic matter in the sample was taken into consideration (Figure 10). *Lepidostoma* varied significantly among treatments, with AC being significantly greater in abundance compared to all other treatments based on LSD. Similar results were obtained for biomass.

Some species had higher densities and biomass on conifer derived detritus, and not alder detrtus. For instance, the collector chironomids *Chaetocladius* and *Heterotanytarsus* were significantly more abundant in conifer-only treatments (Table 2). Others such as *Microspectra, Polypedilum, Rheotanytarsus, Stilocladius,* and *Corynoneura* also showed trends of being more abundant in conifer-only treatments. The nemourid stonefly *Malenka* also tended to be more abundant for the conifer-only treatments. As these were early instars, their biomass did not show any trend to support these findings.

TABLE 2: Common species that were significantly different with regard to treatments with alder versus conifer-only treatments based on ANOVA.

Genera	Measure	Presence or absence of alder	Block	Interaction term	LSD
Zapada	abundance	$P = 0.05$	$P = 0.004$	$P = 0.03$	LMC – higher in alder containing treatments
Zapada	biomass (mg)	$P = 0.04$	$P = 0.02$	$P = 0.07$	LMC – higher in alder containing treatments
Lepidostoma	abundance	$P = 0.01$	$P < 0.001$	$P = 0.02$	LMC – higher in alder containing treatments
Lepidostoma	biomass (mg)	$P = 0.005$	$P < 0.001$	$P = 0.008$	LMC – higher in alder containing treatments
Brillia	abundance	$P < 0.001$	$P = 0.02$	$P = 0.03$	LMC & UMC – higher in alder containing treatments
Brillia	biomass (mg)	$P < 0.001$	$P = 0.02$	$P = 0.02$	LMC – higher in alder containing treatments
Chaetocladius	abundance	$P = 0.03$	$P < 0.001$	$P = 0.01$	LMC - higher in conifer-only treatments
Heterotanytarsus	abundance	$P = 0.02$	$P = 0.001$	$P = 0.05$	LMC – higher in conifer-only treatments
chironomid pupae	abundance	$P = 0.04$	$P < 0.001$	$P = 0.059$	LMC – higher in alder containing treatments

A.



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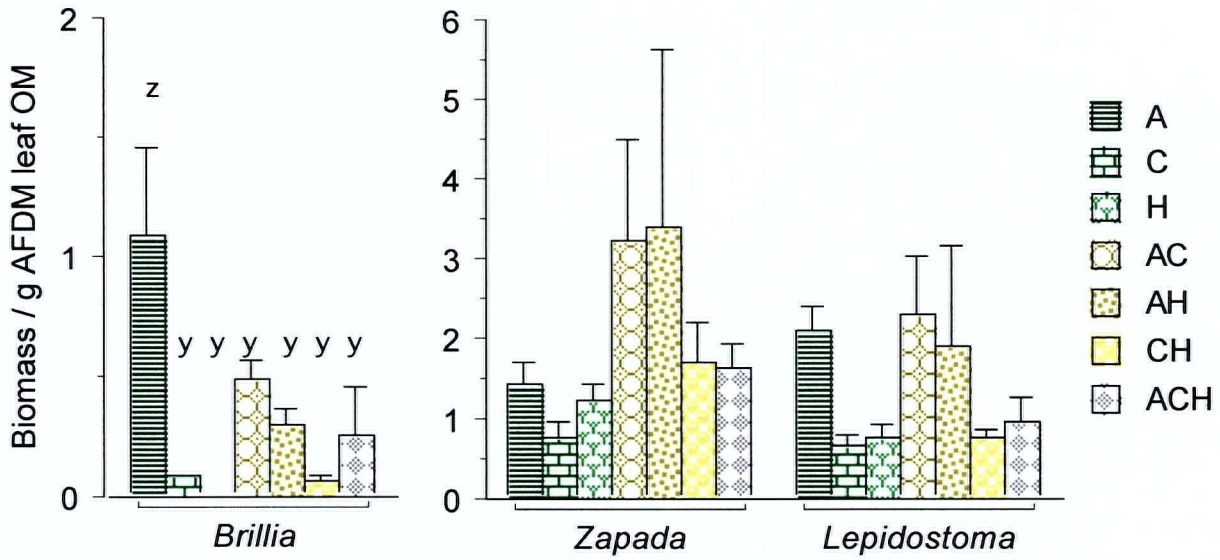


FIGURE 10: Comparison of the means (+1SE) for different shredder species abundance and biomass per unit AFDW in the samples for LMC. *Brillia* abundance (P = 0.002) and biomass (P = 0.02) differed between treatments where bars with different letters are significantly different from each other based on LSD.

The only other group to show a significant effect of treatment were the nematodes, which were significantly higher in the A treatment in LMC (ANOVA: treatment $P = 0.02$, block $P = 0.009$, treatment*block $P < 0.001$). No similar trend was evident in UMC (Figure 11).

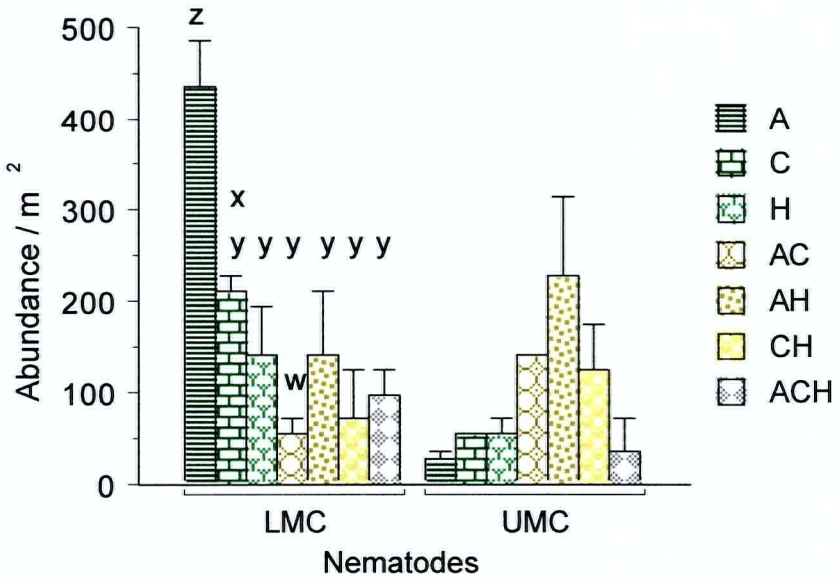


FIGURE 11: Comparison of the means (+1SE) for nematode abundance in LMC and UMC. Nematode abundance differed between treatments where bars with different letters are significantly different from each other based on LSD.

e. Community structure

Principal components analysis (PCA) was employed to determine if the community composition differed between treatments for LMC on day 84. PC axis 1 was based predominantly on the separation between shredder-detritivores and collector-gatherer species, and accounted for 43% of the variation in the 12 genera included (Figure 12). On day 84, the communities had a prominent shredder group with *Zapada* and *Lepidostoma* representing the majority. The community separated out into two groups on the 1st PC, and PC 2 into the alder-containing treatments that had more shredder-detritivore species (*Brillia, Lepidostoma* and *Zapada*) versus the prevalence of collectors such as the chironomids *Microspectra, Chaetocladius, Orthocladius,* and *Microtendipes*, as well as the trichopteran *Wormaldia*, which were more prevalent in the conifer-only treatments. PC axis 2 and PC axis 3 accounted for 18% and 16% of the variation in community structure, respectively. The PCA showed that AC and A treatments have proportionally more shredders (e.g. *Lepidostoma, Zapada*, and *Brillia),* while the others had a higher proportion of collector species. The AH treatment had variable results with the replicates far apart.

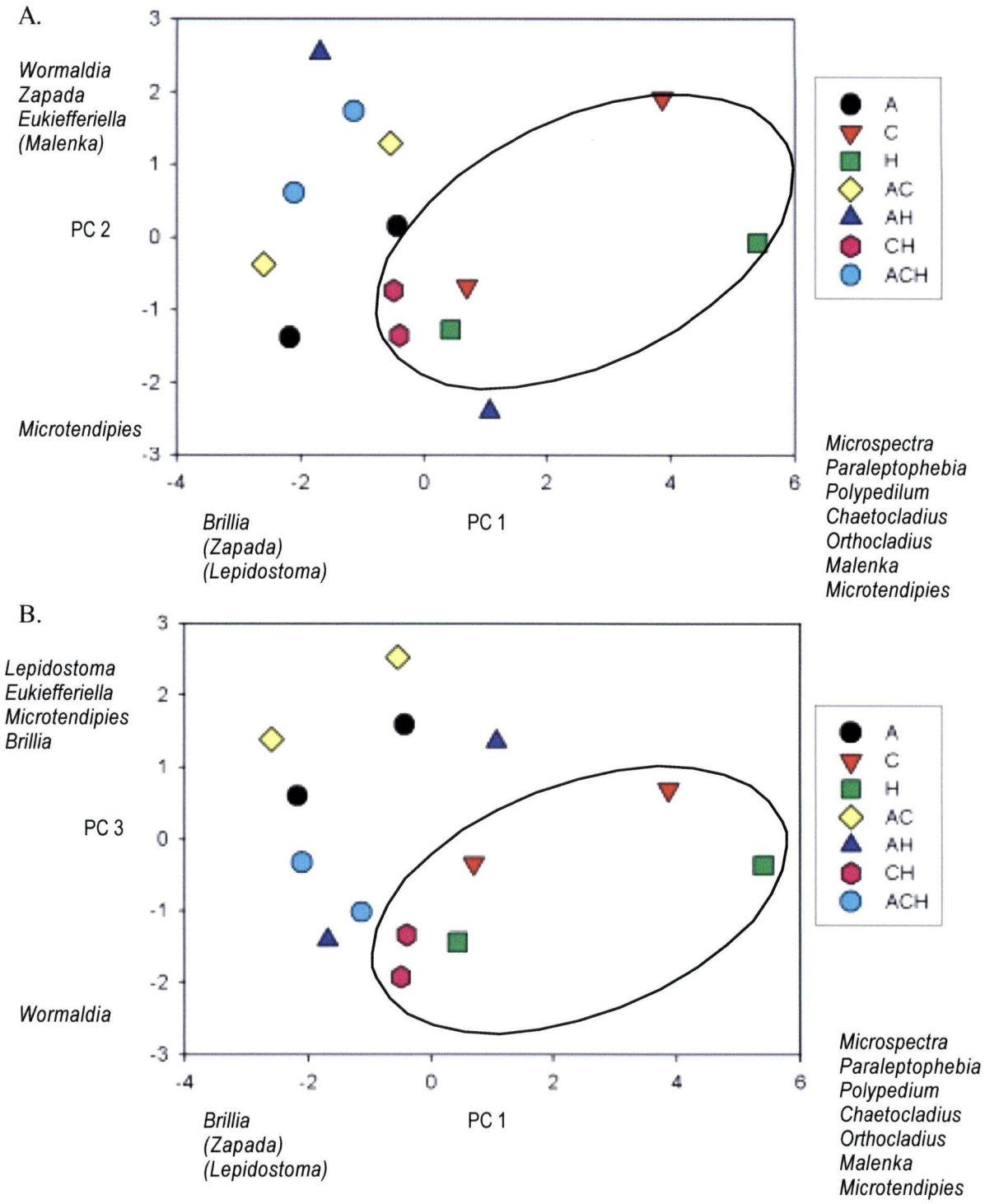
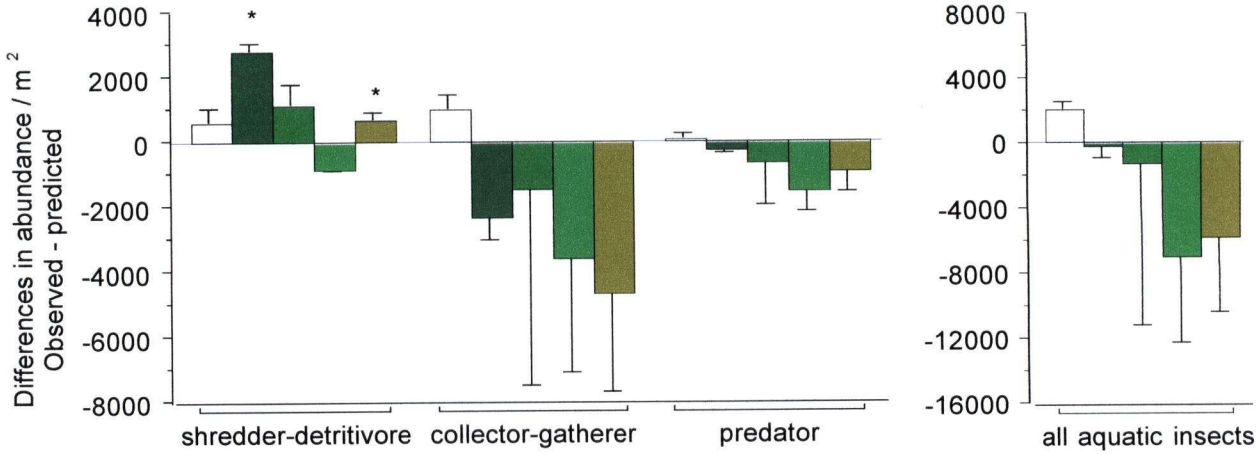


FIGURE 12: Comparison of community structure for day 84 in LMC based on 12 more prevalent species. Species in parentheses are those whose correlation coefficient was below 0.4 but still above the threshold 0.3 value. An ellipse is drawn around the conifer-only treatments to indicate separation from the treatments that include alder.

f. Mixed-litter effects

In LMC, non-additive effects of the observed mixed-litter treatments relative to the predicted value (average of the same species of single-litter treatments) were most evident for shredder-detritivore species. The shredder-detritivores showed a significantly higher density in the AC treatment that was not predictable based on the single species treatments. There was a 43% greater abundance of shredder-detritivores in the mixed AC treatment than the average of the A and C treatments alone (t-test: $P = 0.003$). Shredder-detritivore biomass had similar results with a 62% greater biomass in the observed AC treatment compared to the predicted values (t-test: $P = 0.04$). The abundance of shredder detritivores was also 17% higher in the ACH combination (t-test: $P = 0.05$). In comparison, collector-gatherers were 47% less abundant for ACH treatments; although not significant, these resulted in only a slightly lower relative biomass (Figure 13). There were also trends of lower abundance and biomass in the CH mixed-litter combination.

A.



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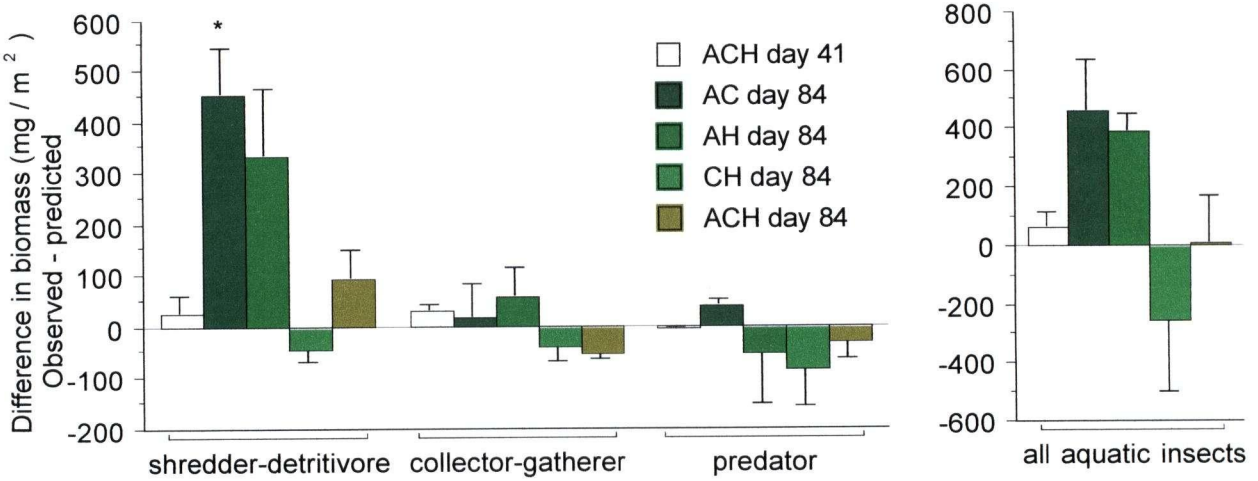

FIGURE 13: Results of multi-litter species treatment residuals for LMC (mean observed – predicted +1SE) on invertebrate data collected on day 84. Day 41 data was included for the three-way combination (ACH). Bars represent the difference between observed and predicted values. Above zero indicates a higher than anticipated result in the mixed-litter treatments, while below zero indicates a lower than predicted result. Panel A shows the species abundance and B shows biomass.

In LMC, the more prevalent collector-gatherers and shredder-detritivores species had the strongest effect on the overall differences in abundance and biomass of aquatic insects in the multi-litter treatments for day 84. The overall increase in biomass of the aquatic insects was primarily based on the shredder-detritivores *Zapada* and *Lepidostoma* while the decrease in abundance was

due to a decrease in collector-gatherers (*Microspectra, Polypedilum, Microtendipes* and *Chaetocladius*). *Lepidostoma* was 50% more abundant in AC compared to predicted values (t-test: P = 0.04) that was similar for biomass (t-test: P = 0.06). *Lepidostoma* biomass was also 46% lower than predicted in the CH mixed-litter (t-test: P = 0.004). These results were not significant when examined on a per gram organic matter basis. *Zapada* showed the largest observed increase in biomass relative to the predicted values when the amount of organic matter in the sample was considered (Figure 14).

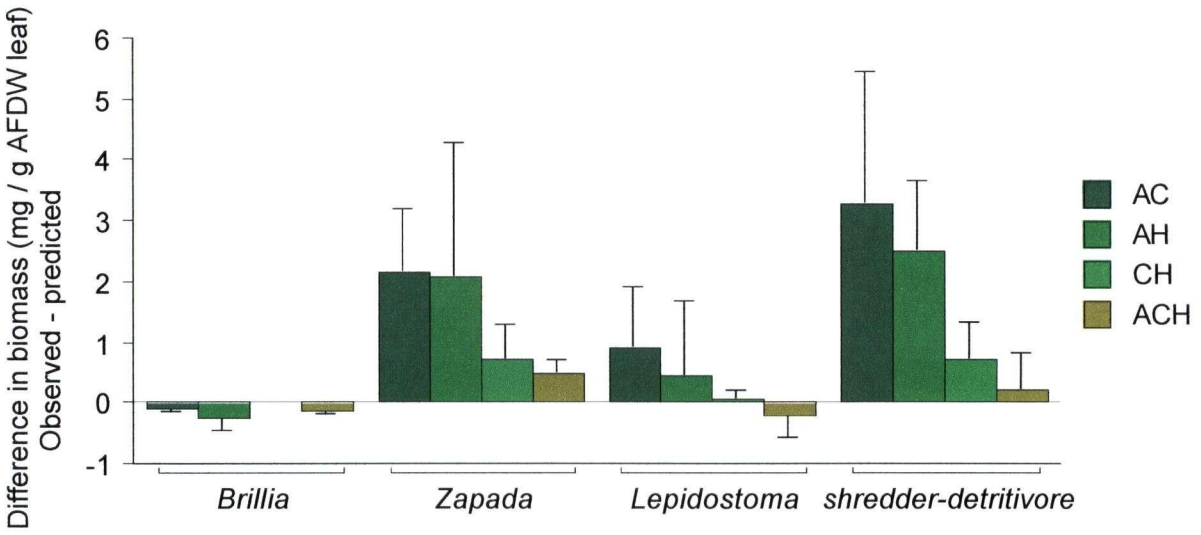

FIGURE 14: Multi-litter species treatment residuals for day 84 (mean +1SE of observed-predicted) for biomass per gram AFDW in the samples. Bars represent the difference between observed and predicted values. Above zero indicates a higher than anticipated result due to the combination treatments, while below zero indicates a lower than predicted result.

When relative size of shredder species was considered, differences were observed between treatments. The most prominent of which was in the shredder *Zapada* spp, particularly *Zapada cinctipies* (Figure 15). Furthermore, the size of *Zapada* spp. had significant interaction terms (ANOVA: $P \leq 0.03$). Additional analysis of LMC data showed that *Zapada* spp. were 33% and 23% larger in AC and AH treatments, respectively. This was based on comparisons between observed and predicted values of an individual insect's weight (t-test: P = 0.01, P = 0.05

respectively). This accounted for part of the trend of higher overall biomass in AC and AH when all aquatic insects were considered, and was not reflected in numbers of insects (Figure 5). No treatment-related difference in average size was evident for other shredder species. The effect of treatment on *Zapada* size in UMC was not evident. *Zapada* was slightly larger in UMC, but not significantly different for the single A and the ACH treatments, compared to the others.

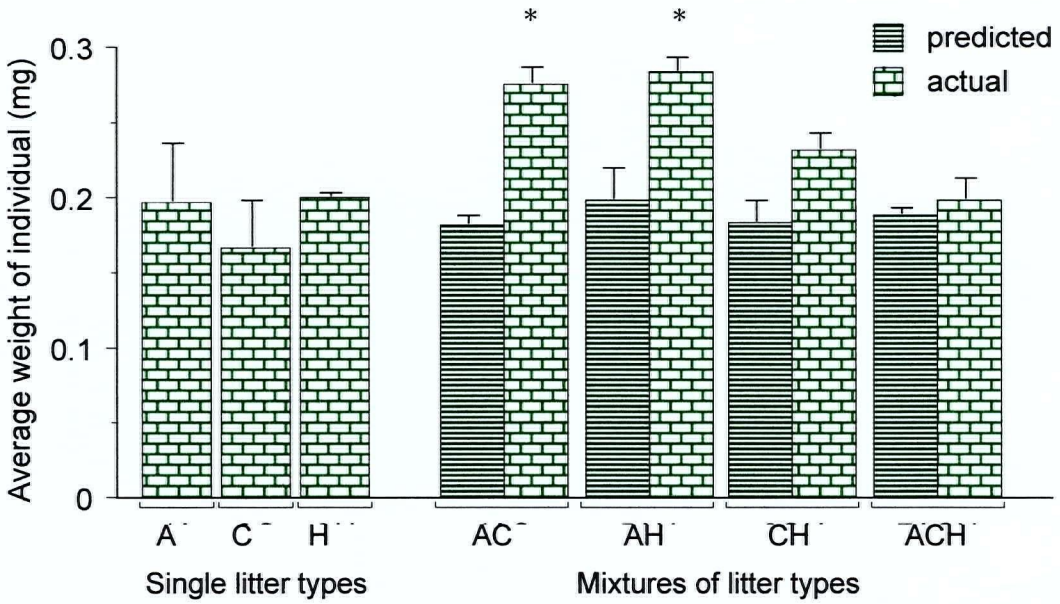

FIGURE 15: Comparison of the size (mean weight +1SE) of the shredder *Zapada* spp. in different treatments at day 84 of the experiment in LMC. For mixtures, * indicates a significant difference between observed and predicted values (t-test).

v. Fungal and invertebrate relations

When fungal and shredder-detritivore biomass was considered in a simple regression, no general association or relationship was evident between ergosterol content (μg/g leaf) and shredder-detritivore biomass (mg/g leaf).

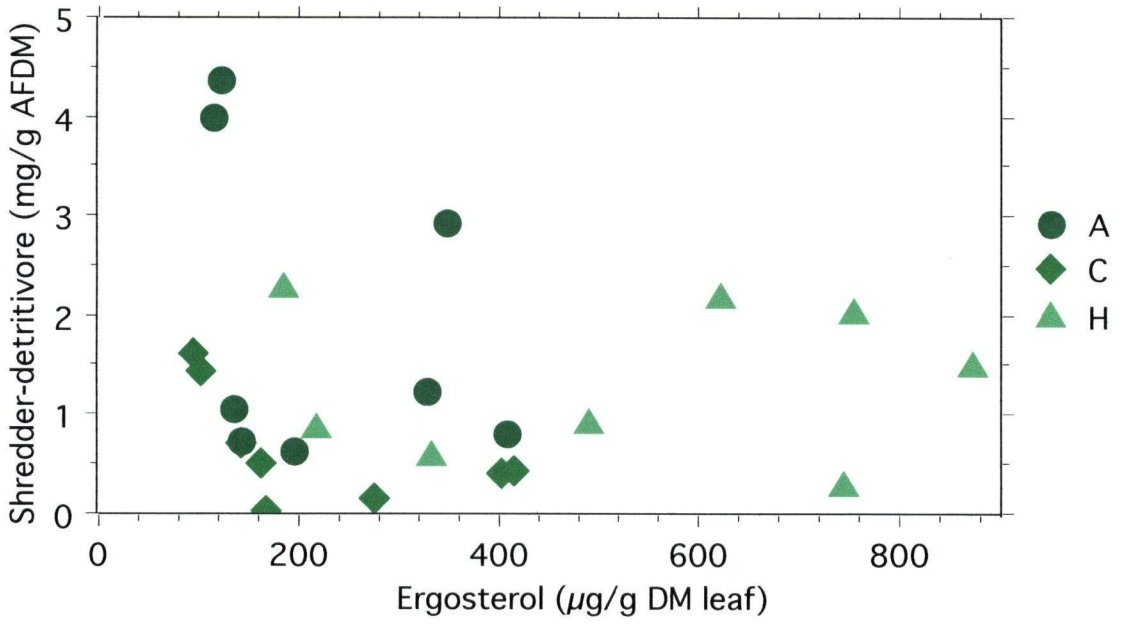

FIGURE 16: Scatterplot of shredder and fungal biomass values for single leaf-litter treatments in both UMC and LMC.

DISCUSSION

The individual species of leaf-litter and the combined litters had large, sometimes contrary, effects on fungal and macroinvertebrate colonization. Our results expand on current views of leaf-litter interactions with stream biota (Graça 2001). Herein we demonstrated that fungal biomass accrual on leaf-litter was not always predictable based on litter decomposition rates. Contrary to expectations, conifer leaf-litter could attain larger total amounts of ergosterol than faster-decaying deciduous species. Placing leaf-litter in mixtures adds another dimension to the development of the macroinvertebrate community structure that has been largely unexplored in stream systems (Sweeney and Vannote 1986, McArthur *et al.* 1994). We showed that, when leaf-litter species were present in mixtures, fungal dynamics and detritivore communities were affected. However, the response of detritivorous macroinvertebrates was not explained simply by fungal biomass accrual on the litter or to a higher diversity of leaf-litter species. The resultant elevated detritivore-shredder abundance and biomass in specific leaf-litter mixtures was indicative of a facilitative effect.

Responses to different single leaf-litter species

The leaf species, as well as colonization time in the stream, are known to affect the amount of fungal biomass present (Suberkropp and Klug 1976, Gessner and Chauvet 1994). Although alder leaves predictably had the fastest fungal biomass accrual rate, hemlock had the highest amount of fungal biomass. This was unanticipated, as it did not correspond with its processing category. Both conifer species were considered slow decay species, while alder has previously been described as a fast to medium decay-rate species depending on the season (Richardson *et al.* 2004). Hemlock had the overall greatest accumulation of fungal biomass with a peak ergosterol concentration of 810 μg/g detritus. Previous literature has documented high levels of ergosterol in leaf-litter packs that allowed invertebrate access, but only for fast decaying deciduous species such as tulip-poplar (*Liriodendron tulipifera* - maximum of 800 μg/g detritus - Suberkropp *et al.* 1993) and ash

(*Fraxinus excelsior* - maximum of 890 μg/g detritus - Gessner and Chauvet 1994). The initial slow and subsequent high accumulation may be attributed to a thick cuticle or other physicochemical characteristic of the hemlock litter along with little invertebrate feeding on the resource. Conifer species can contain phenolic compounds that inhibit fungal growth (Bärlocher & Oertli 1978) and thick waxes or cutins create a physical barrier to fungal degradation (Bärlocher *et al.* 1995). Detritivore-shredder species, particularly the chironomid *Brillia retifinis,* were notably scarce in the hemlock treatments. This lower abundance of shredders would also allow the fungal biomass to increase, as there would be less consumption of the resource. Furthermore, the hemlock litter did not reach a definited maxima of ergosterol within the duration of the study, as did the alder and cedar litter. This pattern of accrual has been found in other slow decay-rate litter species such as oak (*Quercus ilex*), where the ergosterol content increased at a steady, albeit lower rate, and reached a lower maxima before declining at 24 weeks (Gessner and Chauvet 1994). Obtaining higher values of ergosterol in hemlock was unlikely past the 12 week duration of our study, as values were at the upper range of ergosterol content found in leaf-litter species studies without nutrient enrichments (Grattan II and Suberkropp 2001, Gessner and Chauvet 1994). This demonstrates that the fungal component was different for the individual litter species and did not correspond to their processing categories.

Our leaf-litter decay results were contrary to a previous fall study from LMC showing that cedar decayed faster than hemlock (Richardson *et al.* 2004). Our percentage remaining data may not be an accurate representation of what happened in-stream as shredders such as *Lepidostoma* and later instar *Zapada* were excluded from the leaf packs due to mesh size restrictions. The *Lepidostoma* restrictions may not have influenced decay-rates *per se* as this cased-caddis species uses conifer needles as case-building material and not necessarily as a food resource. This mesh restriction did not affect the community composition and biomass results as litter was placed freely in the channels.

Fungi are an integral component in the decay process yet their relative contribution to leaf decay varies in the system depending on environmental and biotic factors (Gessner 1999). For

hemlock litter, there was low shredder abundance relative to alder and cedar, making fungi an important element in hemlock decay. Fungal establishment also appeared to be the foremost contributor in the biotic decomposition of cedar litter in UMC. No difference in percent litter remaining between mesocosms was evident despite significant differences in shredder biomass. Overall, this suggests that lower detritivore shredding activity in UMC was compensated for by higher microbial decay of the litter.

Leaf-litter type affected the resulting macroinvertebrate community composition with the detritivores, particularly shredders, being the most prominently affected in the mesocosms. Higher overall macroinvertebrate biomass in alder-containing versus conifer-only litter treatments was evident in LMC. Culp and Davies (1985) also found higher abundance in substrate patches with alder versus hemlock detritus. This effect was expected as alder has been considered a preferred food source due to its high N content and palatability (Webster and Benfield 1986). This effect was primarily due to the increased presence of shredder species in the alder-containing treatments. Collector-gatherers showed a trend of increased abundance in the single-species conifer litter, but not biomass in LMC. Grouping the species did not disclose what was occurring in the community. At the genus level certain taxa, such as the Orthocladiinae chironomids *Chaetocladius* and *Heterotanytarsus,* showed significant preference for conifer-only treatments.

Although there was higher abundance and biomass of all shredders present in alder litter compared to cedar or hemlock litter, the chironomid shredder *B. retifinis* appeared to be the most sensitive to changes in leaf-litter type. *B. retifinis* showed the strongest preference for alder litter. *B. retifinis* was absent or scarce in the hemlock-only treatment by week 12 suggesting an aversion to feeding on hemlock even when the ergosterol content was highest. Their presence in low numbers at week 6 may indicate a change in the relative composition of fungal species colonizing the hemlock, in the palatability of parts of the litter-fungi complex, or in the nutritional needs of the insect. Summerbell and Cannings (1981) found that Douglas-fir needles were readily consumed by *B. retifinis* before that of cedar. Our data showed that *B. retifinis* was more abundant in cedar than hemlock suggesting that cedar is a better resource than hemlock for *B. retifinis*. This nutritional

difference in the physically similar conifer needles of Douglas-fir and hemlock has been shown in bacterial growth response to leachates from these same species (McArthur and Richardson 2002).

The expected relationship between shredders and leaf-litter was based on the assumption that shredders do not specialize on specific leaf-litter types, but consume any litter type that is adequately modified by microbes (Cummins *et al.* 1989). We found some evidence of this, but it was dependent upon the type of leaf-litter species present. Overall, increases in fungal biomass were not the primary determinant of shredder use of litter as no positive relation between shredders and ergosterol content was evident. For instance, despite the high ergosterol content in hemlock leaf-litter, shredders did not use the resource proportionately to fungal biomass. Although shredders have been found to discriminate between patches of fungal species growing on the litter, no relationship between shredder selection of fungi has been found based on the ability of the fungi to degrade the leaf or specific polysaccharides within the leaf (Suberkropp *et al.* 1983). This supported our contention that it was more than the amount or degree of microbial modification that made leaf-litter palatable to shredders.

Evidence for non-additive consumer responses to mixed leaf-litter

Consumer-resource dynamics of leaf-litter species in combinations were different from that of single leaf-litter species. Macroinvertebrate shredder species exhibited inhibitory or facilitative, non-additive effects depending on the leaf-litter species present. In LMC, an increase in shredder-detritivore larvae biomass in the alder-cedar and alder-hemlock combinations was strongly indicative of a facilitative effect. This larger biomass occurred through a higher abundance of *Lepidostoma* spp. and *Zapada cinctipies*. The *Z. cinctipes* were also 28% larger in size in the alder-conifer combinations. This was ecologically relevant given that by this sampling date, *Z. cinctipies* would have attained 20% of their maximum adult size (Richardson 2001). This difference in adult size has the potential to result in a modification of individual fitness as small females generally produce fewer eggs than large females (Gilbert 1984, Sweeney 1984). In Alaskan streams, *Zapada* spp. have also been found at higher densities in a regenerating dense alder forest with a mixture of

conifer species compared to streams with conifer-dominated or recent clearcut riparian areas (Hernandez *et al.* 2005). Our results also suggested an inhibitory trend in the cedar-hemlock combination. This may explain the lack of shredder response to the treatments when all three litter species were present as multiple interactions would be present.

Changes in shredder growth rates have been evident in previous field studies that manipulated leaf-litter quality. A comparison of leaf-litter treatments found differences in adult stonefly biomass in spring seeps, but this variation was strongly related to the temperature gradient between sites (Sweeney and Vannote 1986). In an alder litter and nutrient [N and phosphorus (P)] addition experiment, Robinson and Gessner (2000) found higher abundance and biomass for nemourid stonefly shredders on fertilized alder litter. In this nutrient addition experiment, no difference was evident in the amount of fungal biomass present. Increases in fungal growth may have been offset by the increase in feeding activity by the abundant shredder population (Robinson and Gessner 2000). Laboratory experiments also showed that litter with higher microbial biomass can produce greater growth rates in detritivores. However, this result was dependent upon environmental variables such as temperature (Ward and Cummins 1979). In our study, temperature was the same for both sets of mesocosms and cannot account for the differing results from the leaf-litter treatments.

The two mesocosms had contrasting results with LMC responding to treatments and the UMC showing little or no response. The mesocosms were significantly different for the majority of species present with the exception of mayfly abundance. LMC had over twice the amount of macroinvertebrates while total fungal biomass accrual was half of that in UMC. Since there was no difference in temperature between the two sets (see Appendix), the higher abundance of macroinvertebrates in LMC may be attributed to the higher water flow rates or periphyton growth due to full sunlight exposure. Increases in the abundance in LMC could be attributed to decreased rates of emigration (Richardson 1991), or conversely, increased rates of emigration in UMC. Differences in fungal biomass accrual patterns could be attributed to the differences in abundance of shredder-detritivores or related to sunlight exposure. Water chemistry and supply of organisms

was presumed to be relatively similar for both mesocosms as the water was diverted from the same undisturbed stream approximately 1 km apart.

One potential mechanism causing the facilitative effect on shredders may be through a modification of fungal growth in the presence of certain leaf-litter combinations, resulting in higher quality resource patches for shredders. The idea of shredder facilitation mediated through fungal establishment has been suggested in other studies investigating shredder species interactions. Jonsson and Malmqvist (2000) suggested that areas with greater shredder species richness had an increased rate of leaf decomposition through either mechanical breakdown of the leaves or indirectly through microbial organisms. Further field research by Jonsson *et al.* (2001) confirmed their laboratory findings and suggested niche complementation as the reason for the increased decomposition rates in high shredder richness areas. Their findings, however, did not rule out facilitation mediated through the microbial trophic level. The facilitative effect on larval growth only occurred in areas of higher shredder invertebrate abundance (LMC - 1643 individuals m^{-2}), and may be related to the interactions that occurred as a result of shredder feeding on the fungal-leaf complex. This does not contradict density-dependent growth found in laboratory studies with shredder species (Cummins *et al.* 1973) as food availability was not thought to be limiting due to the moderate input rate selected for the litter additions (Richardson 1991).

Further evidence for the mechanism involving an interaction between shredders and the leaf-fungal complex was evident in the fungal biomass accrual found in mixed leaf-litter combinations. Here alder reached a 3-fold higher ergosterol content by day 24 when present in a mixture with conifer litter that was unanticipated. Within the same stream, it was unlikely that changes in fungal species colonizing the alder litter could account for such a large increase in ergosterol content (Gessner and Chauvet 1993, Suberkropp *et al.* 1993, Bärlocher and Graça 2002). We conclude that the increase in ergosterol content could only have been from an increase in fungal hyphae growth on the litter.

The change in fungal accrual patterns in mixed-litter compared to single-litter treatments could potentially be mediated through differences in nutrient dynamics of the leaf-litter. Previous

studies have found effects on nutrients in mixed leaf-litter combinations. Terrestrial decay-rate studies on mixed-litter packs have found a greater initial release of N and lower subsequent N immobilization in mixed-litter packs (Blair *et al.* 1990). Interactions between alder and other leaf-litter types leading to facilitative effects on decomposition have also been demonstrated (Fyles and Fyles 1993, McTiernan *et al.* 1997). Sharing of resources between alder and other litter types was proposed to occur through nutrient translocation by fungal hyphae and diffusion (McTiernan *et al.* 1997). In lotic systems, interactions between fast and slow decomposing leaf-litter species have been shown to have an effect on bacterial growth through leachates (McArthur *et al.* 1994). Fungi on decomposing litter in streams can obtain a significant proportion of their nutrients from the water passing over the leaf (Suberkropp and Chauvet 1995, Suberkropp 1998b). Thus, it is conceivable that different leaf species combinations affect fungal growth through leachates and/or via nutrient transfer along fungal hyphae.

Terrestrial forest studies have demonstrated non-additive increases in decay-rates of litter mixtures. As was the case in our study, this effect was only evident when a large invertebrate decomposer community was present (Blair *et al.* 1990, Kaneko and Salamanca 1999). Thus, at high shredder population densities, the presence of cedar or hemlock with alder litter provided a synergistic or facilitative effect for shredders conceivably through increased nutrient availability and retention via fungal hyphae utilizing two different types of nutritional resources in combination.

Some alternate suggestions for the facilitative response by shredders may be interrelationships with variables other than shredder density. There is the potential that the increase in shredders was due simply to the presence of alder in the treatments as a key litter species for facilitative effects to occur. Alder has been noted as a preferred food resource for shredders over other deciduous species such as birch, willow and poplar (Irons *et al.* 1988). Field studies have also noted the importance of alder litter on the invertebrate community with regards to increased abundance and biomass (Hernandez *et al.* 2005). Another possibility was that the effect may be associated with alder when present in high sunlight environments or at specific proportions relative to other litter present. Salamanca *et al.* (1998) have found differences in decay-rates of litter

depending on the proportion of leaf-litter species in the mixtures. This suggests that the alder was present in the two species mixed-litter combinations at proportions that promoted shredder growth and potentially limited competitive interactions. This could also account for the apparent lack of effects when the three species were combined as the amount of alder was lower relative to the conifer species.

Combinations of leaf-litter were not consistently additive. Specific combinations of leaf-litter produced strong non-additive effects on the detrital macroinvertebrates present. These non-additive effects were evident in two species combinations of alder and conifer leaf-litter where shredder-detritivore abundance and size increased. These results suggest that the alder-cedar and alder-hemlock combinations created an enhanced environment that promoted an increase in growth rates. Mixing deciduous and conifer leaf-litter species created more food resource variability and structural habitat complexity than the single species types (Kaneko and Salamanca 1999). The combination of the two conifer species also suggested inhibitory effects of mixing. These results demonstrate that the non-additive effects found in terrestrial detrital systems on detritivore communities (Gartner and Cardon 2004) were also evident in stream systems.

Further exploration of the non-additive effects on shredder species is desirable to confirm the mechanism for these effects and expand on the understanding of the facilitative or inhibitory effects of litter mixtures. As changes in the stream environmental variables (water chemistry or temperature) have a greater effect on fungal growth than that of different leaf-litter species (Chauvet *et al.* 1997), it is important to determine if the non-additive effects on shredders are mediated through fungi, and how much they vary across environmental gradients. The non-additive effects on shredder abundance and biomass could translate to a change in decay-rates of the litter as suggested by the increasing trend in alder litter decay when in leaf-litter mixtures. The restriction of larger detritivores from the leaf packs could have influenced these results and a modified mesh bag design is needed to eliminate this restriction. Potential directions for further research on the effects of mixtures on stream systems also include expansion of the types of leaf-litter examined.

Conclusion

Observations in terrestrial environments indicate that the non-additive effects on decay-rates resulted from mixtures of leaf-litter species in detrital systems that were also evident in the decomposer community > 50% of the time (see Gartner and Cardon 2004 for review). These effects are not limited to terrestrial systems, but are also evident in aquatic environments as indicated in this study. In streams, leaf-litter mixtures are known to have seasonal non-additive effects on decomposition (Swan and Palmer 2004). We expand this knowledge by demonstrating that leaf-litter mixtures also affected biotic variables, such as shredder-detritivore abundance and growth. These non-additive effects have implications for research in mixed canopy streams where results from single-litter species may present a biased view of the actual system productivity.

A potential mechanism for the non-additive effect was an interaction of shredder feeding on the fungal-leaf complex that affected the fungal growth and/or nutritional value when different leaf-litter species were present in mixtures. This change in fungal growth could potentially account for the resultant increase in shredder biomass. However, this facilitative effect only became evident when shredder density was high enough, relative to available resources, to demonstrate a distinct response. This mechanism has also been suggested in terrestrial studies that demonstrate nutrient translocation can occur in leaf mixtures that were potentially mediated through the microbial biomass particularly when in association with fauna abundance (Chapman *et al.* 1988, Salamanca *et al.* 1998).

Leaf-litter species composition of mixtures had a pronounced effect on the aquatic system that was not related to leaf species richness *per se* (McArthur *et al.*1994, Yanoviak 1999, Swan and Palmer 2004). Our results support the hypothesis that changes in riparian forests composition, particularly the alder species, can lead to changes in productivity and structure of benthic communities (Richardson *et al.* 2004, Hernandez *et al.* 2005). In the Pacific Northwest, this is particularly relevant in high gradient headwater streams for the downstream movement of food resources to fish bearing areas (Piccolo and Wipfli 2002, Hernandez *et al.* 2005).

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APPENDIX

FUNGAL BIOMASS PROCESSING PROCEDURE

Fungal biomass index determination for allochthonous inputs in stream mesocosms - based on Gessner & Schmitt (1996) and Dr. Shawn Mansfield, UBC, pers. comm.

Sample preparation:

- Store samples at –20 C until able to process
- Freeze dry (lyophilize) sample for 3.5 to 4 days
- Record total dry weigh (+/- 0.00001g)
- For combinations packs: Separate into different leaf-litter types – A,C,H Record dry weigh (+/- 0.00001g) of different litter types – may omit this if problematic
- Homogenize litter in grinder – use # 40 screen to a sand consistency
- Sub-sample different types of litter into 0.05 g samples for extraction – record weight to 0.00001g (sample must be within 0.049 to 0.053g)

Extraction and saponification in a single reflux cycle:

- Extract lipids with hot alkaline methanol – Extract lipids by reflux in 100 mL of 0.14 M KOH (8 g/L) in pure methanol in Soxhlet extractor for 60 minutes
- Rotovaporate solution to small amount
- Cool liquid to room temperature

Lipid extract cleaned with solid-phase C18 reverse-phase extraction column (Varian Bond Elut C18 3mL column with 500mg sorbent):

- At top of cartridge, add stopcock and 35 mL reservoir
- Condition column with 7.5 mL of conditioning solvent (6 of 0.12 M KOH in methanol plus 1 mL of 0.75M HCl)
- Do not allow column to dry: add 2 mL of conditioning solvent left above column packing – close stopcock
- Transfer extraction flask liquid to column (stopcock closed)
- rinse with 3 mL pure methanol

- Acidify saponified extracts: add 5 mL of 0.75 M HCl - to increase solvent polarity
- Open stopcocks and vacuum manifold - to maintain a constant flow rate
- After sample addition, column washed with 2 mL of 0.4 M KOH in 60% methanol
- Dried in an moderate air flow for 1 hour
- Label and record weight of large vials
- Elute column with 1.4 mL of HPLC grade Isopropanol into vials
- Immediately add 0.3 mL HPLC grade Methanol
- Re-weigh vials

High pressure liquid chromatography (Waters 600E system controller and Waters 700 Satellite WISP):

- Transfer with syringe and microfilter to HPLC vials
- If necessary store at 4°C in sealed vials covered with aluminum foil
- Purified and quantified with high pressure liquid chromatography (HPLC)
- Ergosterol fraction detected by UV absorbance at 282 nm

CHECK LIST OF SPECIES FOUND IN MAYFLY CREEK

Order	Family	Tribe / sub-family	Genera	Trophic relationship[1]	
Tricladida			*Polycelis*		
Nematoda			nematode		
Oligochaeta			worms		
Hyracarina			water mites		
Hyracarina			shrimp		
Araneae			spider		
Beatles			terrestrial beetles /others		
Ostracoda			seed shrimp		
Ostracoda	Isotomidae		spring tails		
Copepoda			copepod		
Ephemeroptera	Ameletidae		*Ameletus*	coll-gath	scraper
Ephemeroptera	Baetidae		*Baetis*	coll-gath	scraper
Ephemeroptera	Baetidae		*Acerpenna*	coll-gath	
Ephemeroptera	Heptageniidae		*Cinygma*	scraper	coll-gath
Ephemeroptera	Heptageniidae		*Epeorus / Ironodes*	coll-gath	scraper
Ephemeroptera	Heptageniidae		*Heptagenia*	scraper	coll-gath
Ephemeroptera	Leptophlebiidae		*Paraleptophlebia*	coll-gath	shred-detr
Ephemeroptera	Ephemerellidae		*Attenella*	coll-gath	
Ephemeroptera	Ephemerellidae		*Drunella*	scraper	
Ephemeroptera	Ephemerellidae		*Serratella*	coll-gath	
Ephemeroptera			ukn. Mayfly	coll-gath	scraper
Plecoptera	Nemouridae	Nemourinae	*Zapada*	shred-detr	
Plecoptera	Nemouridae	Nemourinae	*Podmosta*	shred-detr	coll-gath
Plecoptera	Nemouridae	Nemourinae	*Visoka*	shred-detr	coll-gath
Plecoptera	Nemouridae	Nemourinae	ukn. nemourid	shred-detr	
Plecoptera	Nemouridae	Amphinemurinae	*Malenka*	shred-detr	coll-gath
Plecoptera	Leuctridae		*Perlomyia*	shred-detr	
Plecoptera	Leuctridae		*Despaxia*	shred-detr	
Plecoptera	Leuctridae		ukn. leutrid	shred-detr	

CHECK LIST OF SPECIES - CONTINUED

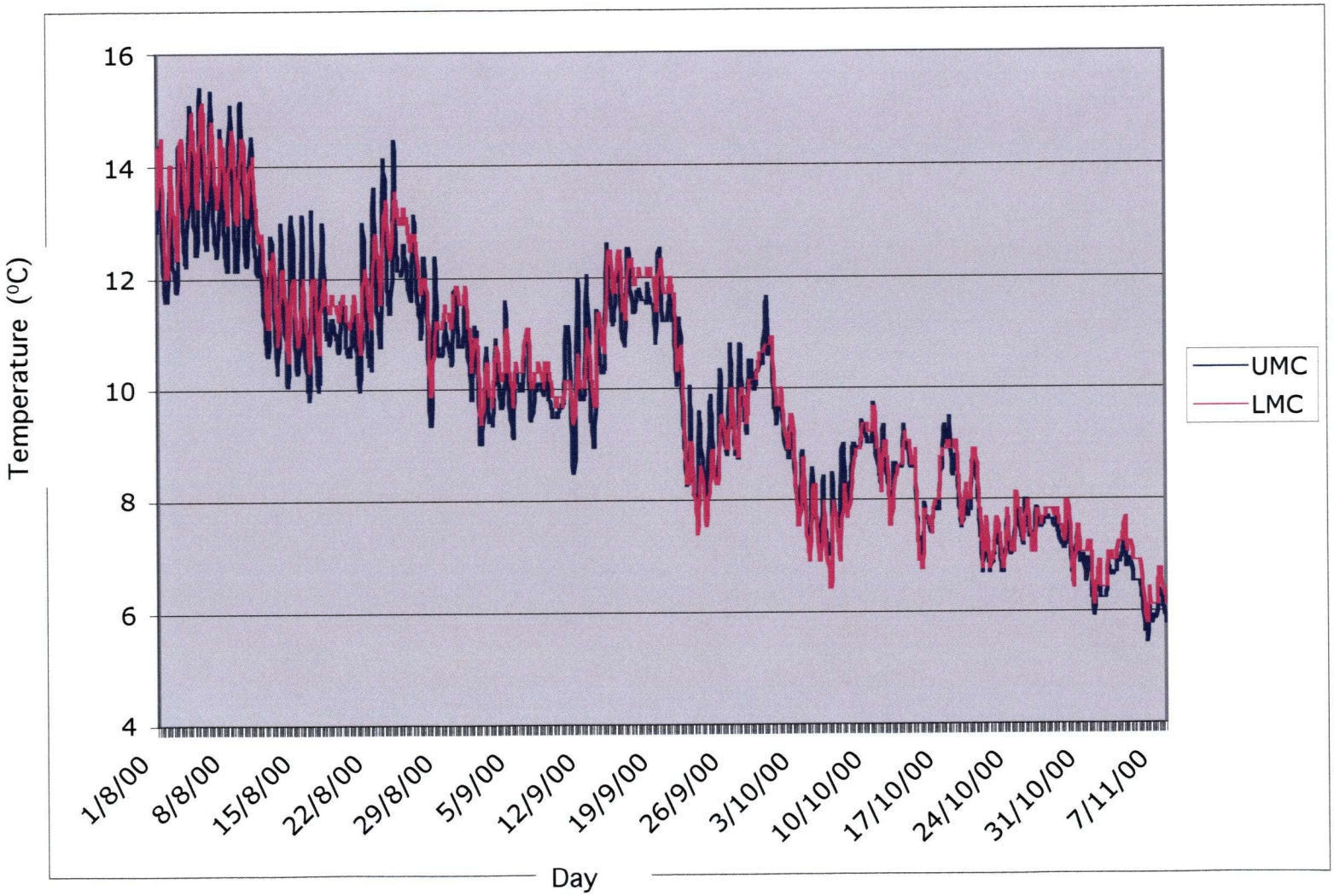
Order	Family	Tribe / sub-family	Genera	Trophic relationship[1]	
Plecoptera	Capniidae		*Paracapnia*	shred-detr	
Plecoptera	Capniidae		*Capnia*	shred-detr	
Plecoptera	Perlodidae		*Arcynopteryx*	predator	
Plecoptera	Chloroperlidae	Chloroperlinae	*Sweltsa*	predator	
Plecoptera	Chloroperlidae	Chloroperlinae	*Plumperla*	coll-gath	predator
Plecoptera	Chloroperlidae	Chloroperlinae	*Suwallia*	predator	
Plecoptera	Chloroperlidae		ukn. chloroperlid	coll-gath	predator
Plecoptera	Chloroperlidae	Paraperlinae	*Kathroperla*	coll-gath	scraper
Trichoptera	Philopotamidae		*Dolophilodes*	coll-filt	
Trichoptera	Philopotamidae		*Wormaldia*	coll-filt	
Trichoptera	Philopotamidae		ukn. philopotamid	coll-filt	
Trichoptera	Polycentropodidae		*Polycentropus*	predator	coll-filt
Trichoptera	Polycentropodidae		*Neureclipsis?*	coll-filt	shred-herb
Trichoptera	Hydropsychidae	Arctopsychinae	*Parapsyche*	coll-filt	
Trichoptera	Hydropsychidae		*Ceratopsyche*	coll-filt	
Trichoptera	Hydropsychidae		*Leptonema*	coll-filt	
Trichoptera	Hydropsychidae		*Homoplectra*	coll-filt	
Trichoptera	Hydropsychidae		ukn. hydropsychid	coll-filt	
Trichoptera	Rhyacophilidae		*Rhyacophila*	predator	coll-gath
Trichoptera	Psychomyiidae		*Lype or Tinodes*	scraper	
Trichoptera	Hydroptilidae		*Agraylea*		
Trichoptera	Lepidostomatidae		*Lepidostoma*	shred-detr	
Trichoptera	Lepidostomatidae		*Micrasema*	shred-herb	coll-gath
Trichoptera	Limnephilidae		*Onocosmoecus*	shred-detr	
Trichoptera	Limnephilidae		*Ecclisomyia*	coll-gath	scraper
Trichoptera	Limnephilidae		*Moselyana*	coll-gath	
Trichoptera			ukn. case caddis	shred-detr	coll-gath
Coleoptera	Elmidae		*Zaitzevia*		
Coleoptera	Hydrophilidae	Hydrochidae	*Hydrochus*	shred-herb	
Diptera	Simuliidae		ukn. black fly	coll-filt	
Diptera	Ceratopogonidae		*Probezzia*	predator	
Diptera	Dixidae		*Dixa*	coll-gath	
Diptera	Chronomidae	Podonominae	*Boreochlus*	coll-gath	scraper

CHECK LIST OF SPECIES - CONTINUED

Order	Family	Tribe / sub-family	Genera	Trophic relationship[1]	
Diptera	Chronomidae	Orthocladiinae	*Brillia*	shred-detr	coll-gath
Diptera	Chronomidae	Orthocladiinae	*Eukiefferiella*	coll-gath	scraper
Diptera	Chronomidae	Orthocladiinae	*Chaetocladius*	coll-gath	
Diptera	Chronomidae	Orthocladiinae	*Parametriocnemus*	coll-gath	
Diptera	Chronomidae	Orthocladiinae	*Orthocladius*	coll-gath	
Diptera	Chronomidae	Orthocladiinae	*Rheocricotpus*	coll-gath	shred-herb
Diptera	Chronomidae	Orthocladiinae	*Psectrocladius*	coll-gath	shred-herb
Diptera	Chronomidae	Orthocladiinae	*Heterotanytarsus*	coll-gath	scraper
Diptera	Chronomidae	Orthocladiinae	*Krenosmittia*	coll-gath	scraper
Diptera	Chronomidae	Orthocladiinae	*Heterotrissocladius*	coll-gath	
Diptera	Chronomidae	Orthocladiinae	*Stilocladius*	coll-gath	scraper
Diptera	Chronomidae	Orthocladiinae	*Krenosmittia*	coll-gath	scraper
Diptera	Chronomidae	Orthocladiinae	ukn orthocladinae	coll-gath	scraper
Diptera	Chronomidae	Tanytarsini	*Corynoneura*	coll-gath	
Diptera	Chronomidae	Tanypodinae	*Pentaneurella*	predator	
Diptera	Chronomidae	Tanypodinae	*Conchapelopia*	predator	
Diptera	Chronomidae	Tanypodinae	*Paramerina*	predator	
Diptera	Chronomidae	Tanypodinae	ukn. tanypodinae	predator	
Diptera	Chronomidae	Chironomini	*Microtendipes*	coll-filt	coll-gath
Diptera	Chronomidae	Chironomini	*Micropsectra*	coll-gath	
Diptera	Chronomidae	Chironomini	*Rheotanytarsus*	coll-filt	
Diptera	Chronomidae	Chironomini	*Polypedilum*	shred-herb	coll-gath
Diptera	Chronomidae	Chironomini	ukn. chronomini	coll-gath	
Diptera	Chronomidae		pupae		
Diptera	Tipulidae	Limoniinae	*Dicranota*	predator	
Diptera	Tipulidae	Limoniinae	*Cryptolabis*	predator	
Diptera	Tipulidae	Limoniinae	*Hesperoconopa*	predator	
Diptera	Tipulidae	Limoniinae	*Hexatoma*	predator	
Diptera	Tipulidae	Limoniinae	ukn tipulid	predator	

[1]Based on two most prominent FFG categories from Merritt and Cummins 1996

TEMPERATURE COMPARISON FOR THE UMC AND LMC MESOCOSMS



AVERAGE ABUNDANCE OF MACROINVERTEBRATES PRESENT IN LMC FOR DAY 84

Species Treatment	Alder	Cedar	Hemlock	Alder-Cedar	Alder-Hemlock	Cedar-Hemlock	Alder-Cedar-Hemlock
Polycelis	2 ± 1.41	1.5 ± 0.35	13 ± 8.49	2.5 ± 1.77	5.5 ± 0.35	1.5 ± 0.35	4 ± 1.41
nematode	25 ± 2.12	12 ± 0.71	8 ± 2.12	3 ± 0.71	8 ± 2.83	4 ± 2.12	5.5 ± 1.06
worms	33.5 ± 5.30	19.5 ± 3.89	29.5 ± 5.30	7.5 ± 0.35	9.5 ± 5.30	21 ± 4.24	173 ± 104.65
water mites	6 ± 2.83	7 ± 0.00	7 ± 1.41	2.5 ± 0.35	1.5 ± 0.35	1.5 ± 0.35	1 ± 0.71
shrimp	1.5 ± 1.06	0 ± 0.00	2 ± 1.41	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00
spider	1 ± 0.71	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00	0 ± 0.00
terrestrial insects	0.5 ± 0.35	2 ± 0.71	0.5 ± 0.35	0 ± 0.00	0 ± 0.00	1.5 ± 1.06	0 ± 0.00
seed shrimp	2 ± 0.00	1.5 ± 0.35	3 ± 0.71	1 ± 0.71	1 ± 0.71	0 ± 0.00	1.5 ± 0.35
spring tails	1 ± 0.71	5.5 ± 3.89	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
copepod	0 ± 0.00	3.5 ± 2.47	0.5 ± 0.35	0.5 ± 0.35	0.5 ± 0.35	1 ± 0.71	0 ± 0.00
Ameletus	4.5 ± 1.77	14.5 ± 6.72	8.5 ± 4.60	21 ± 4.24	11.5 ± 4.60	19.5 ± 3.89	12 ± 0.71
Baetis	21.5 ± 5.30	18.5 ± 3.18	25.5 ± 5.30	12.5 ± 3.18	19 ± 2.12	13.5 ± 0.35	23 ± 0.00
Acerpenna	0.5 ± 0.35	1.5 ± 0.35	1 ± 0.71	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Cinygma	30.5 ± 3.89	44 ± 0.00	43.5 ± 11.67	18.5 ± 6.72	35 ± 5.66	39.5 ± 8.84	41 ± 7.78
Epeorus / Ironodes	11.5 ± 0.35	10 ± 2.12	14 ± 7.07	8.5 ± 1.77	15 ± 5.66	6 ± 0.71	16 ± 1.41
Heptagenia	0 ± 0.00	1.5 ± 1.06	0 ± 0.00	0.5 ± 0.35	1 ± 0.71	1.5 ± 0.35	0 ± 0.00
Paraleptophelbia	14.5 ± 6.01	24.5 ± 6.72	30.5 ± 6.01	10.5 ± 4.60	13.5 ± 2.47	8 ± 2.12	11 ± 2.12
Attenella	0.5 ± 0.35	1 ± 0.00	1.5 ± 0.35	0.5 ± 0.35	1.5 ± 1.06	0 ± 0.00	0.5 ± 0.35
Serratella	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 0.71	0 ± 0.00	0.5 ± 0.35	0 ± 0.00
ukn. Mayfly	9 ± 6.36	1 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Zapada	36 ± 12.02	34 ± 1.41	19.5 ± 3.18	78.5 ± 8.13	70 ± 35.36	20.5 ± 2.47	51 ± 0.71
ukn. nemourid	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 0.00	0.5 ± 0.35	0 ± 0.00
Melenka	15.5 ± 0.35	40 ± 12.02	26.5 ± 4.60	22 ± 2.12	16 ± 0.71	19.5 ± 5.30	22 ± 3.54
Perlomyia	1 ± 0.00	2 ± 1.41	1.5 ± 0.35	1 ± 0.00	1 ± 0.71	1.5 ± 0.35	0 ± 0.00
Despaxia	2 ± 0.00	2.5 ± 0.35	2 ± 0.00	3 ± 0.00	0.5 ± 0.35	0.5 ± 0.35	0 ± 0.00
ukn. leutrid	4 ± 2.83	0.5 ± 0.35	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	1.5 ± 1.06
Paracapnia	4 ± 0.00	4.5 ± 1.77	3.5 ± 1.06	6 ± 0.71	5 ± 1.41	2 ± 0.71	6 ± 0.71
Capnia	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 0.71
Arcynopteryx	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Sweltsa	16.5 ± 0.35	13 ± 3.54	12.5 ± 1.77	5.5 ± 0.35	7 ± 4.95	5.5 ± 0.35	4.5 ± 1.06
Plumperla	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00	0 ± 0.00
Suwallia	0.5 ± 0.35	6.5 ± 4.60	7.5 ± 5.30	3 ± 1.41	3 ± 0.71	0 ± 0.00	2 ± 0.71
ukn. chloroperlid	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	2.5 ± 1.06	0.5 ± 0.35	0 ± 0.00
Kathroperla	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00	0 ± 0.00	0 ± 0.00
Wormaldia	3.5 ± 0.35	8 ± 3.54	7.5 ± 3.18	4 ± 0.71	9.5 ± 4.60	4.5 ± 2.47	12.5 ± 1.06
ukn. philopotamid	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00
Polycenropus	4.5 ± 0.35	9 ± 0.71	5 ± 0.00	4.5 ± 0.35	5 ± 2.83	3.5 ± 1.06	5.5 ± 1.06
Parapsyche	0 ± 0.00	3.5 ± 1.06	0.5 ± 0.35	0.5 ± 0.35	1 ± 0.71	0 ± 0.00	2.5 ± 1.06
Arctopsyche	7 ± 2.83	4 ± 1.41	2 ± 0.71	0 ± 0.00	1.5 ± 1.06	0.5 ± 0.35	5 ± 1.41
Ceratopsyche	3 ± 0.00	1.5 ± 0.35	2.5 ± 1.77	1 ± 0.00	1.5 ± 0.35	1.5 ± 0.35	1.5 ± 0.35
Homoplectra	0 ± 0.00	1.5 ± 0.35	0.5 ± 0.35	0 ± 0.00	0.5 ± 0.35	0 ± 0.00	0 ± 0.00
Diplectrona	0 ± 0.00	0.5 ± 0.35	1 ± 0.71	2.5 ± 1.06	7 ± 4.95	2.5 ± 1.06	6 ± 2.83
Rhyacophila	0 ± 0.00	0.5 ± 0.35	1 ± 0.71	2 ± 0.00	1 ± 0.71	0 ± 0.00	1.5 ± 0.35

AVERAGE ABUNDANCE OF MACROINVERTEBRATES PRESENT IN LMC FOR DAY 84 – CONTINUED

Species Treatment	Alder	Cedar	Hemlock	Alder-Cedar	Alder-Hemlock	Cedar-Hemlock	Alder-Cedar-Hemlock
Stactobiella	0 ± 0.00	0 ± 0.00	1 ± 0.00	1 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Lepidostoma	128 ± 19.80	106.5 ± 16.62	56.5 ± 13.79	235.5 ± 0.35	129 ± 49.50	59 ± 7.78	118.5 ± 6.72
Onocosmoecus	3.5 ± 2.47	1 ± 0.71	0 ± 0.00	2.5 ± 1.06	2 ± 0.71	1 ± 0.71	5.5 ± 2.47
Ecclisomyia	1.5 ± 0.35	2.5 ± 0.35	2 ± 1.41	2 ± 0.71	0 ± 0.00	1 ± 0.00	0.5 ± 0.35
Moselyana	0 ± 0.00	0.5 ± 0.35	1.5 ± 1.06	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00
ukn.case caddis	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00	0 ± 0.00
Ampumixis	1 ± 0.00	2 ± 0.00	0.5 ± 0.35	0.5 ± 0.35	0.5 ± 0.35	1 ± 0.71	1 ± 0.00
ukn. black fly	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	1 ± 0.71	0 ± 0.00	0.5 ± 0.35
Probezzia	3 ± 1.41	5 ± 2.12	3 ± 0.71	1 ± 0.71	2.5 ± 0.35	0 ± 0.00	1 ± 0.71
Boreochlus	0 ± 0.00	2.5 ± 1.77	0.5 ± 0.35	1 ± 0.00	0 ± 0.00	0.5 ± 0.35	0.5 ± 0.35
Brillia	71 ± 0.71	13.5 ± 0.35	0 ± 0.00	54.5 ± 13.08	22 ± 6.36	6.5 ± 2.47	31.5 ± 18.03
Eukiefferiella	26.5 ± 6.72	28 ± 7.07	14 ± 0.71	30.5 ± 8.84	17 ± 2.83	12.5 ± 0.35	25.5 ± 2.47
Chaetocladius	29 ± 0.71	89 ± 4.95	72.5 ± 25.81	36.5 ± 7.42	46.5 ± 1.06	45 ± 4.24	37 ± 10.61
Parametriocnemus	1.5 ± 0.35	0 ± 0.00	0 ± 0.00	5.5 ± 0.35	6.5 ± 1.06	15 ± 2.83	1 ± 0.71
Orthocladius	12 ± 1.41	16 ± 0.00	19 ± 0.71	12.5 ± 0.35	13.5 ± 0.35	9 ± 0.00	9 ± 1.41
Rheocricotpus	22.5 ± 4.60	23.5 ± 3.89	22.5 ± 6.01	29 ± 0.00	21 ± 6.36	21.5 ± 3.18	13 ± 2.12
Psectrocladius	1 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0.5 ± 0.35	0 ± 0.00	0 ± 0.00
Heterotanytarsus	9 ± 1.41	24.5 ± 7.42	26.5 ± 6.01	15 ± 4.95	13 ± 8.49	20 ± 4.24	7.5 ± 1.77
Heterotrissocladius	0.5 ± 0.35	0.5 ± 0.35	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Stilocladius	0.5 ± 0.35	12.5 ± 6.72	3 ± 1.41	4 ± 2.83	8 ± 5.66	3.5 ± 1.06	3.5 ± 0.35
Krenosmittia	0 ± 0.00	0.5 ± 0.35	0.5 ± 0.35	0.5 ± 0.35	0 ± 0.00	0 ± 0.00	0 ± 0.00
ukn orthocladinae	44.5 ± 13.79	23 ± 12.73	26 ± 11.31	21 ± 12.02	10 ± 1.41	4.5 ± 0.35	13.5 ± 3.89
Corynoneura	4.5 ± 1.77	5.5 ± 1.77	6.5 ± 2.47	1.5 ± 0.35	3 ± 0.00	1.5 ± 0.35	2 ± 0.71
Pentaneurella	1.5 ± 0.35	30 ± 7.07	33.5 ± 23.69	42.5 ± 12.37	25 ± 13.44	30.5 ± 3.18	22 ± 4.95
Conchapelopia	19.5 ± 3.18	20.5 ± 8.84	32.5 ± 0.35	0 ± 0.00	0 ± 0.00	0 ± 0.00	3.5 ± 0.35
Paramerina	2 ± 0.71	2.5 ± 0.35	0.5 ± 0.35	0 ± 0.00	1.5 ± 0.35	0.5 ± 0.35	0 ± 0.00
ukn. tanypodinae	49.5 ± 8.84	56 ± 14.85	55.5 ± 2.47	50.5 ± 1.77	36.5 ± 13.79	23.5 ± 3.89	41.5 ± 3.89
Microtendipes	106 ± 4.24	100.5 ± 10.25	102.5 ± 14.50	91.5 ± 11.67	110.5 ± 42.07	81.5 ± 2.47	67.5 ± 3.89
Micropsectra	155.5 ± 28.64	291.5 ± 72.48	322 ± 100.41	143 ± 19.80	183 ± 77.78	196 ± 2.12	89.5 ± 9.55
Rheotanytarsus	4 ± 0.71	9.5 ± 4.60	7.5 ± 1.77	3.5 ± 1.77	2.5 ± 0.35	4.5 ± 2.47	4.5 ± 1.06
Polypedilum	34 ± 13.44	57 ± 14.14	89.5 ± 45.61	35 ± 13.44	47.5 ± 12.37	57 ± 3.54	20.5 ± 1.77
unk. chronomini	1.5 ± 0.35	0.5 ± 0.35	0 ± 0.00	0 ± 0.00	1.5 ± 1.06	0 ± 0.00	0.5 ± 0.35
pupae	40 ± 4.24	26.5 ± 2.47	12 ± 2.83	44.5 ± 10.96	23 ± 5.66	18 ± 4.95	23.5 ± 2.47
Dicranota	1 ± 0.00	2 ± 0.00	1.5 ± 1.06	3 ± 0.00	2.5 ± 0.35	0.5 ± 0.35	1 ± 0.00
Hesperoconopa	5.5 ± 1.06	8.5 ± 1.06	2 ± 1.41	5 ± 0.00	4 ± 0.00	3.5 ± 0.35	1.5 ± 0.35
ukn tipulid	1 ± 0.71	7 ± 2.12	3 ± 0.71	0.5 ± 0.35	2 ± 0.00	2 ± 0.71	2 ± 0.00

AVERAGE ABUNDANCE OF MACORINVERTEBRATES PRESENT IN LMC FOR DAY 84 – CONTINUED

Species Treatment	Alder	Cedar	Hemlock	Alder-Cedar	Alder-Hemlock	Cedar-Hemlock	Alder-Cedar-Hemlock
Genus Richness	55.5 ± 0.35	65.5 ± 3.18	56 ± 7.07	56 ± 3.54	56.5 ± 2.47	50.5 ± 1.77	53.5 ± 0.35
Abundance	1043.5 ± 92.28	1277 ± 198.70	1204.5 ± 324.21	1099.5 ± 102.88	1002.5 ± 216.02	808 ± 31.11	960 ± 97.58
aquatic insect abund	971 ± 86.27	1224.5 ± 186.32	1140.5 ± 315.72	1082.5 ± 100.76	976 ± 206.48	777 ± 31.82	775 ± 3.54
Ephemeropetera	92.5 ± 12.37	116.5 ± 17.32	124.5 ± 35.71	73 ± 21.92	96.5 ± 10.96	88.5 ± 10.96	103.5 ± 10.25
Plecoptera	79.5 ± 14.50	103 ± 14.85	73.5 ± 16.62	119.5 ± 13.08	106.5 ± 27.22	50.5 ± 2.47	88 ± 5.66
Trichoptera	151 ± 14.14	139 ± 15.56	81 ± 20.51	255.5 ± 1.77	158.5 ± 39.24	74.5 ± 6.01	159 ± 4.24
Coleoptera	1 ± 0.00	2 ± 0.00	0.5 ± 0.35	0.5 ± 0.35	0.5 ± 0.35	1 ± 0.71	1 ± 0.00
Diptera	647 ± 73.54	864 ± 169.71	861 ± 243.24	634 ± 64.35	614 ± 183.14	562.5 ± 11.67	423.5 ± 16.62
Chironomidae	636 ± 71.42	841 ± 164.76	851.5 ± 239.36	624 ± 62.93	602 ± 184.55	556.5 ± 11.67	417.5 ± 15.91
Orthocladiinae	218 ± 16.97	231 ± 33.23	184.5 ± 51.97	209 ± 13.44	158 ± 13.44	137.5 ± 5.30	141.5 ± 5.30
Tanytarsini	4.5 ± 1.77	5.5 ± 1.77	6.5 ± 2.47	1.5 ± 0.35	3 ± 0.00	1.5 ± 0.35	2 ± 0.71
Tanypodinae	72.5 ± 4.60	109 ± 31.11	122 ± 21.92	93 ± 14.14	63 ± 26.87	54.5 ± 7.42	67 ± 1.41
Chironomini	301 ± 47.38	466.5 ± 94.40	526 ± 165.46	275 ± 46.67	355 ± 138.59	344.5 ± 3.89	183 ± 11.31
Tipulidae	8 ± 0.71	18 ± 2.83	6.5 ± 3.18	9 ± 0.71	8.5 ± 0.35	6 ± 0.00	4.5 ± 0.35
shredder-herbivore	28.75 ± 4.42	40.25 ± 9.02	56 ± 25.81	32 ± 6.72	34.5 ± 9.55	39.25 ± 3.36	16.75 ± 0.18
shredder-detritivore	229 ± 0.71	190 ± 6.36	112.5 ± 23.69	371 ± 4.95	234.5 ± 14.50	102 ± 6.01	215.75 ± 1.59
scraper	80.5 ± 13.08	92.5 ± 23.33	82.75 ± 23.51	68 ± 18.03	65 ± 10.25	61.5 ± 9.90	72 ± 7.07
predator	102.75 ± 6.54	156.25 ± 37.65	155 ± 33.59	114.75 ± 17.15	88.5 ± 32.53	68 ± 7.78	82.5 ± 0.00
collector-filterer	72.75 ± 5.83	83.25 ± 1.59	75.25 ± 15.38	59.5 ± 9.55	82.25 ± 10.08	56.5 ± 4.60	69 ± 1.77
collector-gatherer	417.25 ± 51.44	635.75 ± 118.62	647 ± 196.58	392.75 ± 55.33	448.25 ± 123.92	431.75 ± 14.32	295.5 ± 2.47

AVERAGE BIOMASS OF INSECTS PRESENT IN LMC FOR DAY 84

Species Treatment	Alder	Cedar	Hemlock	Alder-Cedar	Alder-Hemlock	Cedar-Hemlock	Alder-Cedar-Hemlock
Biomass	50.48 ± 1.02	52.08 ± 3.94	43.27 ± 12.41	77.59 ± 8.70	69.44 ± 3.62	32.93 ± 2.01	48.95 ± 1.39
Ephemeropetera	4.26 ± 0.72	5.46 ± 0.47	5.39 ± 2.25	6.23 ± 3.52	10.04 ± 0.31	3.05 ± 0.01	4.21 ± 0.64
Plecoptera	7.59 ± 1.02	8.41 ± 0.78	11.63 ± 5.52	25.51 ± 4.17	22.60 ± 9.39	7.35 ± 0.99	12.89 ± 0.86
Trichoptera	17.44 ± 2.20	18.17 ± 0.47	11.03 ± 1.84	21.85 ± 3.33	16.90 ± 1.86	7.58 ± 1.96	16.77 ± 0.31
Coleoptera	0.17 ± 0.00	0.19 ± 0.08	0.09 ± 0.06	0.50 ± 0.35	0.03 ± 0.02	0.66 ± 0.47	0.10 ± 0.00
Diptera	19.71 ± 1.03	17.82 ± 1.51	13.62 ± 2.12	20.88 ± 1.60	16.54 ± 3.61	10.62 ± 0.24	13.73 ± 1.57
Chironomidae	18.96 ± 1.20	15.91 ± 2.18	13.24 ± 1.95	18.53 ± 0.61	15.91 ± 3.59	10.46 ± 0.25	12.76 ± 1.73
Orthocladiinae	7.78 ± 0.58	3.97 ± 0.22	2.50 ± 0.72	6.40 ± 0.75	4.27 ± 0.61	2.16 ± 0.03	4.18 ± 1.28
Tanytarsini	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tanypodinae	2.89 ± 0.06	2.79 ± 0.90	2.70 ± 0.30	2.45 ± 0.00	2.12 ± 0.88	1.71 ± 0.00	2.26 ± 0.07
Chironomini	6.60 ± 0.73	8.56 ± 1.07	7.76 ± 0.99	7.88 ± 0.65	8.65 ± 3.18	6.31 ± 0.12	5.72 ± 0.38
Tipulidae	0.25 ± 0.02	1.67 ± 0.79	0.21 ± 0.13	2.18 ± 1.10	0.45 ± 0.09	0.16 ± 0.00	0.79 ± 0.25
shredder-herbivore	0.50 ± 0.07	0.42 ± 0.11	0.36 ± 0.14	0.50 ± 0.00	0.44 ± 0.08	0.29 ± 0.04	0.22 ± 0.04
shredder-detritivore	18.65 ± 1.09	12.53 ± 0.32	7.49 ± 1.78	41.87 ± 3.87	32.45 ± 6.68	7.39 ± 0.28	18.25 ± 1.43
scraper	5.05 ± 0.65	4.44 ± 0.40	3.47 ± 1.37	4.66 ± 1.32	5.94 ± 0.42	3.92 ± 0.79	3.14 ± 0.80
predator	4.45 ± 0.07	7.52 ± 0.24	10.64 ± 5.23	8.17 ± 0.36	4.39 ± 1.63	4.17 ± 0.61	5.66 ± 0.15
collector-filterer	6.96 ± 0.03	13.66 ± 3.19	10.35 ± 1.40	6.39 ± 1.51	10.10 ± 0.86	7.46 ± 2.40	11.93 ± 0.94
collector-gatherer	13.20 ± 1.41	12.95 ± 0.81	10.70 ± 2.55	14.21 ± 2.13	15.26 ± 1.65	9.41 ± 0.53	9.16 ± 0.07

AVERAGE BIOMASS OF INSECTS PRESENT IN LMC FOR DAY 84 -CONTINUED

Species Treatment	Alder	Cedar	Hemlock	Alder-Cedar	Alder-Hemlock	Cedar-Hemlock	Alder-Cedar-Hemlock
Ameletus	1.30 ± 0.48	2.03 ± 0.78	1.52 ± 0.75	2.62 ± 0.38	3.33 ± 0.59	3.66 ± 0.70	1.24 ± 0.72
Baetis	0.72 ± 0.24	1.44 ± 0.73	0.96 ± 0.27	0.27 ± 0.10	1.09 ± 0.07	1.17 ± 0.27	1.49 ± 0.08
Acerpenna	0.14 ± 0.10	0.17 ± 0.11	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cinygma	0.72 ± 0.08	1.39 ± 0.05	1.01 ± 0.47	0.45 ± 0.16	1.20 ± 0.04	1.03 ± 0.26	1.09 ± 0.16
Epeorus / Ironodes	2.36 ± 0.64	1.10 ± 0.12	2.30 ± 1.49	1.90 ± 1.27	3.35 ± 1.44	0.60 ± 0.13	1.06 ± 0.32
Heptagenia	0.00 ± 0.00	0.05 ± 0.03	0.00 ± 0.00	0.94 ± 0.66	2.30 ± 1.63	0.01 ± 0.01	0.00 ± 0.00
Paraleptophelbia	0.21 ± 0.10	1.22 ± 0.18	0.84 ± 0.04	2.26 ± 1.04	2.02 ± 0.66	0.10 ± 0.01	0.52 ± 0.27
Attenella	0.03 ± 0.02	0.09 ± 0.02	0.28 ± 0.06	0.06 ± 0.04	0.08 ± 0.06	0.00 ± 0.00	0.06 ± 0.04
Serratella	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.25	0.00 ± 0.00	0.15 ± 0.10	0.00 ± 0.00
ukn. Mayfly	0.09 ± 0.06	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Zapada	6.46 ± 1.41	5.75 ± 0.95	3.88 ± 0.61	21.66 ± 2.86	20.27 ± 10.43	4.73 ± 0.43	10.09 ± 0.66
ukn. nemourid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
Melenka	0.10 ± 0.01	0.24 ± 0.11	0.18 ± 0.07	0.35 ± 0.09	0.18 ± 0.02	0.12 ± 0.02	0.22 ± 0.04
Perlomyia	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.06 ± 0.04	0.01 ± 0.01	0.27 ± 0.07	0.00 ± 0.00
Despaxia	0.20 ± 0.10	0.31 ± 0.18	0.10 ± 0.01	0.17 ± 0.05	0.11 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
ukn. leutrid	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.12
Paracapnia	0.05 ± 0.00	0.06 ± 0.03	0.07 ± 0.03	0.23 ± 0.08	0.14 ± 0.02	0.03 ± 0.02	0.12 ± 0.01
Capnia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.28 ± 0.20
Arcynopteryx	0.00 ± 0.00	0.00 ± 0.00	5.41 ± 3.83	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sweltsa	0.72 ± 0.29	1.96 ± 0.17	1.91 ± 0.93	2.87 ± 1.12	0.71 ± 0.50	2.09 ± 0.71	2.00 ± 0.16
Plumperla	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.26 ± 0.18	0.00 ± 0.00	0.00 ± 0.00
Suwallia	0.02 ± 0.02	0.07 ± 0.05	0.07 ± 0.05	0.17 ± 0.11	0.21 ± 0.11	0.00 ± 0.00	0.01 ± 0.00
ukn. chloroperlid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.59 ± 0.33	0.10 ± 0.07	0.00 ± 0.00
Kathroperla	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Wormaldia	1.81 ± 0.08	4.34 ± 1.74	4.18 ± 1.78	0.77 ± 0.04	4.17 ± 1.40	1.91 ± 1.20	4.11 ± 0.34
ukn. philopotamid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Polycenropus	0.12 ± 0.01	1.56 ± 0.71	0.29 ± 0.13	0.24 ± 0.04	0.46 ± 0.28	0.34 ± 0.13	0.74 ± 0.41
Parapsyche	0.00 ± 0.00	4.06 ± 1.30	1.94 ± 1.37	1.21 ± 0.85	0.67 ± 0.48	0.00 ± 0.00	3.05 ± 0.59
Arctopsyche	0.63 ± 0.29	0.38 ± 0.11	0.14 ± 0.05	0.00 ± 0.00	0.10 ± 0.07	1.94 ± 1.37	0.49 ± 0.16
Ceratopsyche	1.73 ± 0.06	0.59 ± 0.20	0.86 ± 0.61	0.52 ± 0.11	0.66 ± 0.01	0.64 ± 0.16	0.73 ± 0.44
Homoplectra	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Diplectrona	0.00 ± 0.00	0.03 ± 0.02	0.09 ± 0.06	0.35 ± 0.17	0.67 ± 0.47	0.18 ± 0.05	0.70 ± 0.37
Rhyacophila	0.00 ± 0.00	0.01 ± 0.01	0.08 ± 0.05	0.44 ± 0.21	0.25 ± 0.18	0.00 ± 0.00	0.15 ± 0.07

AVERAGE BIOMASS OF INSECTS PRESENT IN LMC FOR DAY 84 - CONTINUED

Species Treatment	Alder	Cedar	Hemlock	Alder-Cedar	Alder-Hemlock	Cedar-Hemlock	Alder-Cedar-Hemlock
Stactobiella	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.07	0.29 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lepidostoma	9.09 ± 0.16	5.28 ± 1.03	2.73 ± 1.05	15.40 ± 1.40	9.49 ± 4.06	2.14 ± 0.09	5.64 ± 0.18
Onocosmoecus	0.33 ± 0.24	0.06 ± 0.04	0.00 ± 0.00	0.94 ± 0.50	0.36 ± 0.06	0.02 ± 0.02	0.56 ± 0.38
Ecclisomyia	3.72 ± 2.60	1.81 ± 0.95	0.51 ± 0.36	1.69 ± 1.08	0.00 ± 0.00	0.40 ± 0.26	0.60 ± 0.43
Moselyana	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
ukn.case caddis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
Ampumixis	0.17 ± 0.00	0.19 ± 0.08	0.09 ± 0.06	0.50 ± 0.35	0.03 ± 0.02	0.66 ± 0.47	0.10 ± 0.00
ukn. black fly	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.04	0.00 ± 0.00	0.03 ± 0.02
Probezzia	0.51 ± 0.14	0.24 ± 0.12	0.17 ± 0.04	0.17 ± 0.12	0.12 ± 0.04	0.00 ± 0.00	0.16 ± 0.11
Boreochlus	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Brillia	4.64 ± 0.72	0.65 ± 0.03	0.00 ± 0.00	3.65 ± 0.95	1.63 ± 0.43	0.16 ± 0.02	2.05 ± 1.30
Eukiefferiella	0.52 ± 0.13	0.47 ± 0.09	0.21 ± 0.02	0.42 ± 0.11	0.32 ± 0.11	0.15 ± 0.00	0.43 ± 0.03
Chaetocladius	0.77 ± 0.07	1.37 ± 0.19	1.03 ± 0.40	0.52 ± 0.12	0.80 ± 0.21	0.61 ± 0.11	0.68 ± 0.14
Parametriocnemus	0.06 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.00	0.14 ± 0.02	0.39 ± 0.03	0.03 ± 0.02
Orthocladius	0.36 ± 0.04	0.41 ± 0.01	0.47 ± 0.01	0.32 ± 0.03	0.40 ± 0.05	0.27 ± 0.04	0.38 ± 0.10
Rheocricotpus	0.88 ± 0.13	0.65 ± 0.17	0.47 ± 0.15	0.83 ± 0.06	0.70 ± 0.16	0.42 ± 0.08	0.34 ± 0.09
Psectrocladius	0.04 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Heterotanytarsus	0.10 ± 0.05	0.16 ± 0.04	0.13 ± 0.04	0.13 ± 0.06	0.08 ± 0.05	0.10 ± 0.02	0.07 ± 0.01
Heterotrissocladius	0.04 ± 0.03	0.04 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stilocladius	0.01 ± 0.01	0.05 ± 0.03	0.01 ± 0.00	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.00	0.04 ± 0.00
Krenosmittia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ukn orthocladinae	0.36 ± 0.18	0.17 ± 0.10	0.18 ± 0.11	0.38 ± 0.04	0.15 ± 0.02	0.06 ± 0.00	0.17 ± 0.00
Corynoneura	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pentaneurella	0.17 ± 0.07	0.45 ± 0.12	0.68 ± 0.48	0.63 ± 0.09	0.57 ± 0.33	0.61 ± 0.01	0.52 ± 0.14
Conchapelopia	0.41 ± 0.10	0.34 ± 0.09	0.45 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.02
Paramerina	0.05 ± 0.02	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
ukn. tanypodinae	2.26 ± 0.24	1.96 ± 0.68	1.56 ± 0.12	1.82 ± 0.10	1.53 ± 0.55	1.09 ± 0.00	1.66 ± 0.09
Microtendipes	5.43 ± 0.57	6.87 ± 0.80	5.90 ± 0.65	6.80 ± 0.65	7.07 ± 2.90	5.21 ± 0.01	4.89 ± 0.31
Micropsectra	1.05 ± 0.17	1.36 ± 0.30	1.57 ± 0.20	0.90 ± 0.06	1.38 ± 0.29	0.91 ± 0.11	0.72 ± 0.09
Rheotanytarsus	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
Polypedilum	0.07 ± 0.01	0.20 ± 0.05	0.25 ± 0.13	0.16 ± 0.06	0.17 ± 0.01	0.17 ± 0.00	0.09 ± 0.02
unk. chronomini	0.03 ± 0.00	0.10 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
pupae	1.68 ± 0.06	0.57 ± 0.01	0.27 ± 0.07	1.79 ± 0.50	0.87 ± 0.14	0.28 ± 0.09	0.59 ± 0.00
Dicranota	0.01 ± 0.00	1.15 ± 0.74	0.09 ± 0.06	1.98 ± 1.14	0.31 ± 0.10	0.05 ± 0.04	0.02 ± 0.00
Hesperoconopa	0.22 ± 0.04	0.23 ± 0.02	0.10 ± 0.07	0.17 ± 0.01	0.12 ± 0.01	0.06 ± 0.01	0.15 ± 0.06
ukn tipulid	0.02 ± 0.01	0.28 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.03	0.62 ± 0.32