INVERTEBRATE CONTRIBUTION TO LEAF BREAKDOWN IN PYNN'S BROOK, NL.

by

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The undersigned certify that they have read, and recommend to the Environmental Science Unit (Division of Science) for acceptance, a thesis entitled “Invertebrate Contribution to Leaf Breakdown in Pynn’s Brook, NL” submitted by Johannah Baird in partial fulfillment of the requirements for the degree of Bachelor of Science, Honours.

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ABSTRACT

Leaf bags of fine and coarse mesh were placed at two locations, one with an open tree canopy, the other with a closed tree canopy, in Pynn's Brook on June 30th 2015. Bags were collected after 2, 30, 37 and 44 days. After collection, invertebrates were counted and leaf material remaining was determined to measure leaf breakdown rate. There was no significant difference in leaf mass remaining (*R*) between the two sites. Comparisons between mesh types found a difference in leaf breakdown at two collection days. The difference at 2 days was small (2.7%) and may not be biologically meaningful. At 37 days, the difference was larger (8.41%) and may be related to a larger proportion of shredder taxa, seen in coarse mesh bags, or higher absolute numbers of invertebrates. The invertebrate community was dominated by *Diptera* spp. across all collection days and mesh types, but after 37 days, communities in coarse mesh bags had a higher proportion of shredder orders than did fine mesh bags.

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LITURATURE REVIEW

Decomposition

The process of decomposition occurs in terrestrial and aquatic environments. Decomposition can be defined as any change that occurs in organic matter that is aged and later dies (Brinson et al. 1981). Within decomposition there are processes of decay, leaching and immobilization. Decay is the loss of organic matter through respiration and assimilation by microbes and detritus feeders (Brinson et al. 1981). Leaching includes loss of soluble inorganic or organic materials. Immobilization is the conversion of inorganic compounds to organic compounds. An opposing process to immobilization is mineralization, the conversion of elements from organic forms to inorganic states through microbial activity (Soil Science Glossary Terms Committee 2008). Inorganic ions are lost by mineralization and leaching during decomposition and through immobilization are converted to organic matter (Brinson et al. 1981).

Decomposition in terrestrial systems is of interest to soil ecologists describing soil related problems. In soil studies it is accepted that decomposition depends on the organisms present for processing, the chemical nature of the material and the availability of nitrogen. Processing of material is divided into two factors, external and internal factors. External factors include water availability, temperature and presence of fungi and bacteria. The chemical composition of the material is the major internal factor (Petersen & Cummins 1974). These factors of terrestrial systems are also true in aquatic systems. Ninety percent of the global terrestrial plant production enters the dead organic matter pool in both terrestrial and aquatic environments making decomposition and sequestration of nutrients, such as carbon, key components of ecosystem functioning (Gessner et al. 2010).

Decomposition in small headwater streams becomes a prevalent process when stream banks are covered or shaded with surrounding, also called riparian vegetation (Lagrue et al. 2001). A dense tree canopy decreases incoming solar radiation, which reduces the rate of primary production (Bärlocher 1985, Lagrue et al. 2011, Danger et al. 2013). Due to the decrease in primary production, the proportion of energy derived from vegetation entering the stream will increase. Invertebrates and other organisms will rely on twigs, leaves and dissolved organic matter (Bärlocher 1985). This indicates that not all stream communities depend on in-stream primary production as a food source (Petersen & Cummins 1974), although primary production may stimulate degradation of material by providing high quality resources to detritivores (Danger et al. 2013). The combined effects of primary production and decomposition have the potential to change ecosystem functioning (Danger et al. 2013).

Input of Material to Stream Systems

Leaves are the main detrital input to streams, and are an important food and energy source for stream organisms such as invertebrates and the rest of the aquatic food web (Bärlocher 1985, Tuchman & King 1993, Irons et al. 1994, Muto et al. 2011). Leaves are an important energy import from the surrounding terrestrial environment (Petersen & Cummins 1974). Leaves naturally enter a stream after abscission, the process of leaf detachment, and accumulation following abscission produces leaf packs (Petersen & Cummins 1974). Leaf resources in streams are generally short-lived which is potentially due to periodic high water flow fragmenting the leaves and flushing the material downstream (Gessner et al. 2010).

Mid-latitude streams have a highly pulsed input of litter occurring over a short autumn period. For example, in a Central Finland stream it was found that 75% of the annual leaf input

occurred over a period of two weeks in late September (Haapala et al. 2001). The pulse in leaf input results in synchronized detritus feeder life cycles or development of alternative feeding strategies (Haapala et al. 2001, Muto et al. 2011). Invertebrate emergence, from egg and pupa, occurs before leaf abscission (Petersen & Cummins 1974), while the feeding and growth periods of larva and adults occur in fall to early winter when high quality leaves are highly available (Petersen & Cummins 1974, Tuchman & King 1993, Haapala et al. 2001, Muto et al 2011). The same synchronization is seen in fungal communities that quickly utilize high quality litter. Utilization is paired with fast growth and reproduction to ensure the completion of fungal life cycles (Gessner et al. 2010).

Leaf Processing

Petersen & Cummins (1974) have devised a processing budget for leaf material that has entered a stream system. Since 1974, much research has been done on leaf processing in streams and the steps outlined by Petersen & Cummins (1974) are now viewed as a more simultaneous and interactive (Haapala et al. 2001). Decomposition by invertebrates, aquatic fungi and bacteria along with physical fragmentation and leaching, are all integral process affecting leaf litter losses (Bergfur et al. 2007).

Once leaves have entered a stream system the first step in decomposition or processing is leaching (Petersen & Cummins 1974). Leaching removes soluble organic and inorganic compounds from the leaves which are then released to the stream and available to be converted into biomass and carbon dioxide (Petersen & Cummins 1974, Brinson et al. 1981, Bärlocher 1985, Muto et al. 2011). Most losses due to leaching occur over the first 24 hours of submersion (Haapala et al. 2001, Bergfur et al. 2007) and cause dramatic weight loss of material (Petersen &

Cummins 1974, Irons et al. 1994). Over the entire process of leaching, approximately two days, about 15 percent of the leaf pack is broken down leaving 85 percent available for further processing (Petersen & Cummins 1974, Haapala et al. 2001).

The remaining leaf pack undergoes further losses through microbial and invertebrate colonization. Microbial colonization of leaves, more recently referred to as conditioning occurs over a two week period, and can contribute significantly to decomposition (Petersen & Cummins 1974). After the two weeks there is an increase in microbial biomass and spore production (Sridhar & Bärlocher 2000), followed by initial invertebrate colonization over a 90 day period. The microbial communities present on leaves act as an intermediate trophic level between leaves present in a water body and invertebrates (Sridhar & Bärlocher 2000). This trophic dynamic is the consequence of leaf material acquiring nitrogen in the form of protein, lignin, microbial cells, enzymes and other secretions by microbes from water flowing over leaves and resulting leaching of soluble material (Bärlocher 1985, Sridhar & Bärlocher 2000). The process of acquiring nitrogen increases palatability and nutrient content and is referred to as conditioning (Bärlocher 1985). Microbes also soften the leaf tissue further conditioning the leaves (Irons et al. 1994).

Conditioning is the result of two microbial processes, production and catalysis. Production is mostly due to microbial reproduction, the microbial cells on their own are nutritious and are eaten by macroinvertebrates along with the leaf material. The microbial community also releases various compounds that may be digestible by invertebrates. Catalysis also involves microbial secretions, but only those involved in the breakdown of structural carbohydrates, which invertebrates are unable to digest. These secretions breakdown the structural carbohydrates and invertebrates may finish the breakdown process once initial steps are complete, or ingest the enzymes, which may remain active inside the animal for some time (Bärlocher 1985).

Conditioning, or simply microbial colonization, makes the leaves more palatable and nutritious for invertebrates (Sridhar & Bärlocher 2000, Muto et al. 2011). Colonization by leaf shredding invertebrates, which feed on the microbial community as well as the leaves themselves increases fragmentation and breakdown (Bergfur et al. 2007, Muto et al. 2011). Invertebrates feeding on leaves select certain species for feeding and show a preference for non-sterile leaves over sterile leaves of the same leaf species (Petersen & Cummins 1974). This preference for one leaf species over another is due to the microbial community and its rate of colonization present on the leaf. Different leaf species have different concentrations of fungal biomass and fungal species richness may regulate shredder consumption of leaf material (Muto et al. 2011).Given that microbial colonization of leaf material increases palatability and nutrition for invertebrates it is thought that microbial communities play a larger role in leaf litter breakdown than previously recorded (Bergfur et al. 2007).

The microbial contribution to processing of leaves is about equal to the processing by invertebrates, although the effect of microbial processing is dependent on the time scale that the measurements are made and the resistance of a leaf species to utilization (Petersen & Cummins 1974). Invertebrate and microbial processing together breakdown an additional 43 percent of the leaf pack, with 24 percent attributable to invertebrates and 19 percent to microbial processing. These percentages do not include any losses due to physical breakdown, for example by abrasion or non-feeding invertebrate activity.

Invertebrate species can be classified into functional feeding groups based on their feeding activity as fine or small particle feeders, scrapers grazing on the microbial community, collector-gathers feeding on fragmented particles, shredders fragmenting and consuming material, and predators feeding on other invertebrates. Each classification contributes differently to the

breakdown of leaf material (Petersen & Cummins 1974, Chauvet et al. 1993, Tuchman & King 1993).

After leaching, conditioning and invertebrate processing, 2-41% of the original leaf mass remains (Petersen & Cummins 1974, Bärlocher 1985, Haapala et al. 2001, Bergfur et al. 2007), depending on differences in processing rates among leaf species. The remaining leaf mass continues to be processed by microbes and invertebrates, and becomes fragmented. These fragments enter a smaller category of detritus which undergoes further breakdown by a set of different organisms at rates differing from those of previous breakdown (Petersen & Cummins 1974).

Quantifying Leaf Breakdown

Leaf breakdown occurs through the process of leaching, conditioning and invertebrate colonization previously described. Unlike decomposition, leaf breakdown also includes fragmentation of leaf material caused by the movement of water and the activity of invertebrates feeding on the leaf material (Camacho et al. 2009, Marano et al. 2011, Gonçalves et al. 2012).

Measurements of leaf weight loss can be made in two ways, one using a linear model and the second using an exponential model, the latter being more widely used (Petersen & Cummins 1974, Brinson et al. 1981, Tuchman & King 1993, Haapala et al. 2001). The exponential model assumes that for any amount of material at any time there is a constant fraction loss. This model uses the parameter k, the leaf pack rate coefficient (Petersen & Cummins 1974).

Leaves fall into three categories of breakdown speed: slow ($k \leq 0.005$), medium ($0.005 < k \leq 0.010$), and fast ($k \geq 0.010$). Leaf species in the fast category that enter a stream in autumn (September to November) will be unrecognizable by April to June, while slow decaying leaves

would require more than 15 months for 90% of the material to be processed. This can also be viewed in terms of how much material would remain after one year: fast species would have <3% of leaf mass remaining, while approximately 16% of the mass of slow species would remain (Petersen & Cummins 1974).

Leaf breakdown can also be quantified by leaf mass remaining or percentage leaf mass remaining (*R*). The most common calculation is done by dividing the mass remaining after a given time period by the initial mass of leaf material (Petersen & Cummins 1974, Tuchman & King 1993, Haapala et al. 2001, Bergfur et al. 2007).

Factors Affecting Leaf Breakdown and Processing

Leaf breakdown varies by species and is influenced among other factors by water temperature, nutrient availability, stream pH, composition of stream biota, microbial abundance, shredder abundance, species diversity and canopy cover (Irons et al. 1994, Haapala et al. 2001, Gessner et al. 2010, Lagrue et al. 2011). Leaf quality, measured in physical and chemical properties, related to nutrient, lignin and tannin content, also affects breakdown rates (Irons et al. 1994, Haapala et al. 2001, Kominoski et al. 2009, Muto et al. 2011). Leaves with high condensed tannin concentration, cellulose or lignin make poor quality litter and break down slowly, while leaves with low tannin concentration and high nutrient content are of higher quality and decompose faster (Irons et al. 1994, Haapala et al. 2001, Gessner et al. 2010, Muto et al. 2011, König et al. 2014). Leaves with low carbon to nitrogen ratio are of higher quality while leaves with high carbon to nitrogen ratio will be lower quality (Kominoski et al. 2009).

Effect of nutrients

Input of nutrients into streams is an important regulator of leaf litter decomposition (Haapala et al. 2001, Bergfur et al. 2007). The rate of nutrient cycling is dependent on mineralization of compounds by organisms decomposing the leaf material and competition for these nutrients with producers (Danger et al. 2013). This input results in increased breakdown rates, which is likely due to increases in microbial processing (Haapala et al. 2001). In contrast, in low nutrient conditions microbe growth is minimized and carbon is expelled, increasing mineralization (Danger et al. 2013). Therefore, under low nutrient conditions decomposition will be low. Microbial communities gain nitrogen from water flowing over the leaves, therefore increases in dissolved nitrogen will result in increases of microbial biomass (Sridhar & Bärlocher 2000). As part of the nutrient effect, conditioning can alter breakdown rate and consumption of leaf material (Bärlocher 1985). Increases in microbial biomass in turn lead to increases in invertebrate abundance and density, although not richness, and corresponding increases in leaf litter breakdown due to high consumption (Bärlocher 1985, Bergfur et al. 2007). High quality leaves such as alder contain more protein then other leaf species and are able to be consumed with little conditioning, while conifer needles, low quality litter, require high levels of conditioning before invertebrates can consume them (Bärlocher 1985).

Effect of Temperature

By compiling temperature regimes and processing coefficients Irons et al. (1994) showed that processing rates are often faster in colder water temperatures than at warmer ones and this may be attributed to microbial and invertebrate communities temperature tolerance. Microbial communities are greatly affected by temperature while less of a response is shown in invertebrate communities (Iron et al. 1994).

Breakdown rates are governed mainly by fungi in tropical and temperate streams, this becomes less important at higher latitudes (Irons et al. 1994, Haapala et al. 2001). Given this, shredders should assume a greater role in high latitude streams, and it is possible that invertebrate feeding may over shadow the role of microbes. Invertebrate abundance on leaf packs showed an increase with increasing latitude, in both invertebrate number and biomass (Irons et al. 1994). Invertebrate communities may play a larger role in tropical streams than previously thought as streams with high invertebrate abundance have shown leaf mass remaining values ranging from 10-80% among leaf species after 28 days (König et al. 2014). The presence or abundance of invertebrates and their role in leaf breakdown in tropical streams is likely dependent on stream characteristics rather than the overall region where the studies are conducted (König et al. 2014).

Irons et al. (1994) suggest three hypothesis for explaining these results. First, invertebrates are evolutionary adapted to the thermal regimes found in cooler waters, and are able to process leaves at temperatures close to 0°C. Thus the processing rates of a leaf species will increase with increasing latitude. Second, microbes are less able to maintain metabolic rates at cooler temperatures, therefore the rate of microbial processing will decrease with increasing latitude. Finally, invertebrates are more important in the colder waters of high latitudes and altitudes (Irons et al. 1994).

Effect of microbial, detritivore and leaf species diversity

Any change in diversity can alter primary production, decomposition and affect nutrient cycles, further affecting ecosystem functioning (Kominoski et al. 2009, Gessner et al. 2010). Climate change, species invasions and the spread of pathogens alter leaf input decomposition (Kominoski et al. 2009). Changes in the leaf species composition and diversity of riparian species alters leaf litter inputs which can cause large changes in leaf species utilization and rates of

decomposition by both microbial and detritivore communities (Kominoski et al. 2009, Gessner et al. 2010). When multiple leaf species are in an area the differences in nutrients among the species allows for communities to optimize uptake potentially altering the decomposition rates between leaf species. Multiple leaf species also provide fungi the opportunity to redistribute leaf decomposing enzymes and nutrients between leaf species so that all leaf species present can be used (Gessner et al. 2010). When mixing of high and low quality leaves occurs it results in increases mass loss (Kominoski et al. 2009).

A wide range in microbial species should improve the efficiency of the microbial community to decompose a range of leaf constituents as bacteria and fungi have complementary enzymes allowing then to better decompose leaf material, enhancing litter decomposition rates (Gessner et al. 2010). Bacterial growth on leaf material is enhanced by the presence of fungi but bacteria can also inhibit fungal growth, therefore the relationship between fungi and bacteria is not always beneficial (Kominoski et al. 2009). The importance of riparian vegetation diversity is also shown in changes of microbial respiration, as a measure of microbial community activity, that occurred when high quality leaf species were mixed with low quality leaf species. This could be a result of changes in activity of the total microbial community or structure (Kominoski et al. 2009).

Detritivore diversity can be enhanced by algal presence with fungal decomposers, resulting in faster decomposition rates and may improve the quality of leaf material. This relationship is normally found under low nutrient conditions (Danger et al. 2013). Because decomposition rates tend to respond to differences in diversity at higher trophic levels, invertebrate richness could result in in faster decomposition of material (Gessner et al. 2010).

Effect of canopy cover

Leaf diversity input can be affected by logging practices which change the amount of incoming solar radiation further influencing microbial and invertebrate communities (Lagrue et al. 2011). The effects of logging practices can be observed in many trophic levels with invertebrate and larger consumer such as fish species changing their dietary habits in logged areas (Glaz et al. 2014). The amount of sunlight reaching a stream can vary depending on stream width, riparian vegetation structure and land use practices. Even small changes in canopy cover can result in large changes in microbial and detritivores ability to decompose incoming material. Open canopy stream reaches tend to have higher leaf breakdown rates than streams with a closed canopy and less incoming light however, this may not always be the case (Lagrue et al. 2011). When starting from low light levels small increases in light intensity can increase litter decomposition but large changes in light intensity can have the opposite effect. Increases in light can accelerate decomposition through increases in microbial enzyme efficiency and shredder feeding activity. Sunlight also stimulates the growth of algae which results in increased availability of carbon, which in turn is linked to higher invertebrate densities found in open canopy areas as algal growth can act as an additional food source for some invertebrates (Lagrue et al. 2001). Invertebrates can congregate on leaf packs in open canopy reaches to avoid predation as the leaf packs acts as a refugia, which may result in faster leaf decomposition rates within these reaches.

Measurement Techniques

Litter bags are the most common way to measure leaf breakdown in water bodies (Muto et al. 2011). However, a more uncommon way is to have loose leaf packs held together by string. Leaves on strings may provide on overestimate of decomposition (Petersen & Cummins 1974).

This is due to the potential loss of large leaf particles that are not completely decomposed. However, measurements of total decomposition may by better suited to the string method as this allows the true effect of large invertebrates (Petersen & Cummins 1974). Petersen & Cummins (1974) suggest that in running water mesh bags would exclude invertebrates. Also, microbial processing of the leaves would not occur due to reduced exchange rates of nutrients and possible anaerobic conditions through the use of oxygen. In contrast to the view of Petersen & Cummins (1974), Muto et al. (2011) suggested placing a wire ring inside the leaf bag, to keep its shape, would allow for increased water flow and access by invertebrates.

Consideration of when a study will take place is important. For example in January there is little leaf material remaining in a stream, only material that takes longer to process will be present in small amounts. Introduction of a litter bag into a stream during this time can result in unrealistic measurements of leaf decay because leaves of high quality are a rare find for the time of year (Petersen & Cummins 1974). Boreal streams have a long cold winter and a warmer short summer. Streams are usually snow and ice covered from November to as long as May (Muto et al. 2011). This is important when determining when to place leaf bags in a stream as well as the collection interval. Muto et al. (2011) found that birch leaf placed in the stream in fall lost between 24-26% of the initial mass and by early spring (approximately 230 days) 42%-69% was lost. The long collection interval was due to ice cover and variation in collection dates could cause differences in the determined rate of leaf breakdown with season. Invertebrate community composition can vary with season with higher total abundance and percentage of shredders found in the fall and early spring than would be found in leaf bags collected in late spring.

The leaves to be placed into litter bags are commonly collected at abscission and air dried for a period of approximately two weeks (Bergfur et al. 2007, Petersen & Cummins 1974).

Haapala et al. (2001) suggest it is possible that the use of air-dried leaves can affect the calculated decay rates, however this is negligible when compared to oven drying at high temperatures. Litter bags can be kept in place using an array of methods. A common method of leaf placement is to attach leaf bags to house bricks, usually weighing two pounds, and then placing bags into the stream (Petersen & Cummins 1974, Sridhar & Bärlocher 2000, Haapala et al. 2001).

When litter bag decomposition studies are conducted an unknown loss of incompletely decayed fragments occurs. This loss is often included in decomposition estimates and the amount depends largely on the mesh size of the litter bag used (Brinson et al. 1981). Litter is usually enclosed in mesh bags of 0.8-10 mm mesh size. Fourteen by eleven centimeter bags with four by eight millimetre mesh opening were used by Haapala et al. (2001) as this size allowed for free passage of all macroinvertebrates present in a body of water. Smaller size mesh bags, for example 0.3 mm, result in slower decomposition rates when compared across the same leaf material to coarse mesh bags of 5 mm (Brinson et al. 1981, Bergfur et al. 2007), because smaller mesh bags exclude detritus feeders. Also, fragmentation of leaf material is less likely to happen when compared to larger mesh sizes (Brinson et al. 1981). The effect of detritus feeders can be measured directly by excluding them from litter bags and comparing the rates of decay with detritus feeders absent and present.

Invertebrates show a preference for leaf species with higher processing coefficients, indicated by a larger *k* value (Petersen & Cummins 1974). Leaf species with higher processing rates are colonized first by microbial communities. There is a continuum of leaf use starting with fast species then continuing with slow and medium species. Fast species are commonly selected over slow species. This continuum results in new sources of food material over time as slow leaves become more palatable and available in a stream. This understanding is reinforced due to

the presence of a period of time in which no invertebrate colonization of leaf packs occurs. Also this period varies among leaf species (Petersen & Cummins 1974). Happala et al. (2001) agree with this continuum concluding that willow leaves, categorized as a medium species by Petersen & Cummins (1974) may play an important role in supporting the shredder population of forest streams due to their slower breakdown rate. Common leaf species in boreal ecosystems include alder, birch and aspen with the latter two species accounting for 50% of total deciduous leaf litter inputs. These species have been shown to have *k* values of 0.0065/day, 0.0053/day and 0.0035/day classifying them as slow, medium and fast species receptively (Muto et al 2011). These differing rates of leaf breakdown provide continuous food for invertebrates when leaf input may be low. Therefore, the choice of leaf species can greatly affect the processing rates observed over varying time scales.

Bergfur et al. (2007) suggest the introduction of leaf litter bags to a stream results in a more palatable food source for invertebrates compared to ambient detritus. This can be explained by the availability of suitable substrate, which provides invertebrates with a place for attachment and protection (Petersen & Cummins 1974, Tuchman & King 1993). Suitable substrate consists of large stable particles greater than 4.75 mm, which retains coarse particulate organic matter. If there is little suitable substrate, the introduction of leaf packs can provide a more suitable habitat, attracting invertebrates (Tuchman & King 1993). The abundance and taxon richness of invertebrates attracted to the leaf pack rapidly increases in early stages of an experiment which may be attributed to leaf packs providing a more suitable substrate or that leaf packs provide a high quality readily available food source (Tuchman & King 1993, Bergfur et al. 2007).

INTRODUCTION

This study examined leaf breakdown in open and closed canopy reaches, using fine and coarse mesh bags to separate the contributions of microbial and invertebrate communities. These leaf bags, were placed in Pynn's Brook stream in an open tree canopy and a closed tree canopy area. The Pynn's Brook area is a pilot study site of the Newfoundland and Labrador Boreal Ecosystem Latitudinal Transect (NL-BELT). NL-BELT consists of four main sites in Western Newfoundland and South-Eastern Labrador that differ only in climatic factors. The project focuses on how forest processes are currently functioning and how these processes may be affected under a changing climate. Sites in the NL-BELT have been precisely logged and are under management providing an opportunity to study the effects of sustainable forest management practices. The current study of leaf decomposition in Pynn's Brook is a pilot study to gain information on leaf decomposition, microbial and invertebrate communities present in the area.

The proposed questions are, does leaf litter breakdown vary between open and closed tree canopy areas, does leaf litter decomposition vary when invertebrates are excluded from litter bags and, will leaf loss be higher in bags that have higher invertebrate abundance? Based on the information presented the proposed hypotheses are, leaf decomposition will be higher in the open canopy area and if invertebrate abundance is higher in coarse mesh bags then leaf mass loss will be higher.

MATERIALS AND METHODS

The study was conducted in Pynn's Brook, NL located between Corner Brook and Deer Lake (Figure 1). Two lengths of the stream also called reaches were sampled, one in an open tree canopy and one in a closed tree canopy area. The open tree canopy area was an upstream location while the closed tree canopy area was a downstream location. Surveys to characterize substrate and surrounding vegetation cover were previously conducted in August of 2013.

Litter bags of both fine and coarse mesh were used. Fine mesh bags had openings of approximately 0.07 mm^2 and coarse mesh bags had openings of 2.25 mm^2. Coarse mesh bags had an area of fine mesh at the bottom, the downstream end, to prevent loss of fragmented material. Three grams of leaf material were placed in each of 27 fine and 27 coarse mesh bags for a total of 54 bags, which used all available leaf material. Air-dried birch leaves collected in the fall before abscission and were used. Filled litter bags were placed in a storage container and re-wetted with distilled water to prevent fragmentation of material during transportation to the field.

Six bags, three fine and three coarse mesh, were placed at three locations in each reach. Groups of three bags were kept in the stream by driving long nails into the stream bed. Bags were placed in the stream on June 30th, 2015. Three bags of each mesh size were randomly collected from each reach on July 2nd, July 30th, August 6th and August 13th 2015, which corresponds to 2, 30, 37 and 44 days in stream. Three fine and three coarse mesh bags were placed in the stream on June 30th and then immediately removed to account for any loss of leaf material due to handling. Litter bags were placed in labeled Ziploc® bags to retain moisture until transported back to the laboratory.

After collection each leaf was placed in a dish of distilled water under a dissecting microscope. Invertebrates were removed from the leaf, by scanning the upper and lower side, and

placed in 70% ethanol until later counting and identification to order. Order was determined following the key presented in Merritt et al. (2008). Initial processing of litter bags occurred within 24 hours after collection. Once all leaves were processed they were dried in an oven at approximately 50°C for 12 hours and then weighed using an analytical balance.

Three HOBO Pendant® temperature/light loggers (Onset Computer Corporation, Bourne MA) were placed on July 2nd 2015 and were collected on August 13th 2015. Two loggers were placed in the open tree canopy reach and one was placed in the closed tree canopy reach. Loggers were kept in place using fishing line tied to stream substrate and surrounding vegetation.

A naturally occur leaf pack was collected from the open canopy reach on August 6th 2015 for analysis of the fungal community present. Leaf material was placed in distilled water in 150ml beakers and was to be agitated using an orbital shaker for 48 hours to stimulate sporulation of aquatic fungi, followed by filtration through a 5μm membrane and staining with Trypan blue for identification (Cornut et al. 2010). Due to a malfunction of the orbital shaker, the analysis of the fungal community could not be completed.

Data Analysis

Leaf mass remaining was calculated using the formula $R = \frac{W(t_f)}{W(t_i)}$ where $W(t_f)$ is the weight of the leaf material after time t and $W(t_i)$ is the initial weight of the leaf material. This calculation provided a direct comparison of breakdown between locations and collection date. Remaining leaf mass was averaged across the three bags of each mesh size collected from both reaches per collection date. R was compared between reaches using independent sample, equal variance t-tests at a 95% confidence interval. The same method was used to compare fine and coarse mesh litter bags.

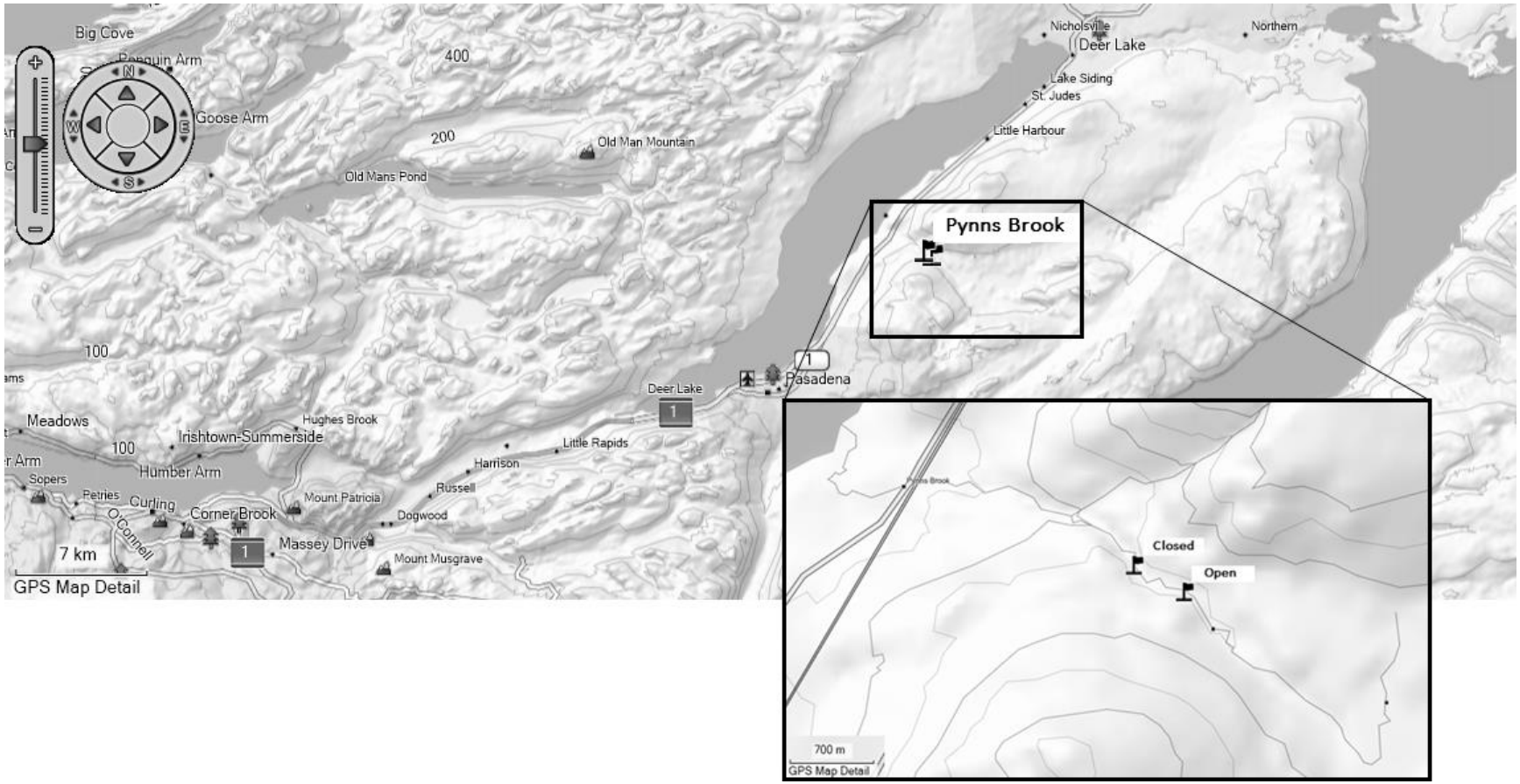

Figure 1: Map of Pynn's Brook showing location of open and closed canopy reaches

RESULTS

Only one of the three loggers was recovered, from the open canopy reach. Due to this, comparisons of temperature and light intensity between reaches were unable to be made. However, the general pattern of temperature variation is likely similar in the two reaches, although actual values may differ due to shading effects. Maximum temperatures occurred between 15:00 and 19:00 h while minimum temperatures occurred between 04:00 and 08:00 h. Average temperatures during collection intervals of 30 June – 20 July, 21 July – 6 August, and 7 – 13 August showed little variation while maximum and minimum temperatures ranged from 16.6 – 17.6°C and from 12.6 – 13.3°C, respectively.

The microbial community was unable to be analyzed from the naturally occur leaf pack as problems occurred with the agitator. Due to time constraints, no data was collected.

T-tests comparing leaf mass remaining (*R*) between open tree canopy and close tree canopy reaches for both mesh types per collection date showed no significant difference in *R* (Table 1). Although the reaches were selected for differences in tree canopy cover, there was little difference in relative cover of coniferous and deciduous species (unpublished data), so direct comparisons of *R* between mesh types were made by pooling values across reaches (Figure 2). There was a significant difference in *R* between mesh types after 2 days ($t = 2.5$, df = 10, $p = 0.031$) and 37 days ($t = 3.9$, df = 10, $p = 0.003$). After two days *R* was 2.7% higher in coarse mesh bags, which is statically significant but may not represent a biologically meaningful difference. After 37 days the difference increased to 8.4% which may be attributed to changes in invertebrate community composition.

Table 1: Summary statistics of equal variance t-tests conducted at a 95% confidence interval testing leaf mass remaining (*R*) in open canopy and closed canopy reaches.

Days in Stream	Mesh Type	t	df	p
2	Fine	-1.524	4	0.202
	Coarse	0.040	4	0.997
30	Fine	0.157	4	0.883
	Coarse	1.326	4	0.255
37	Fine	0.360	4	0.737
	Coarse	0.240	4	0.822
44	Fine	2.29	3	0.106
	Coarse	1.004	4	0.372

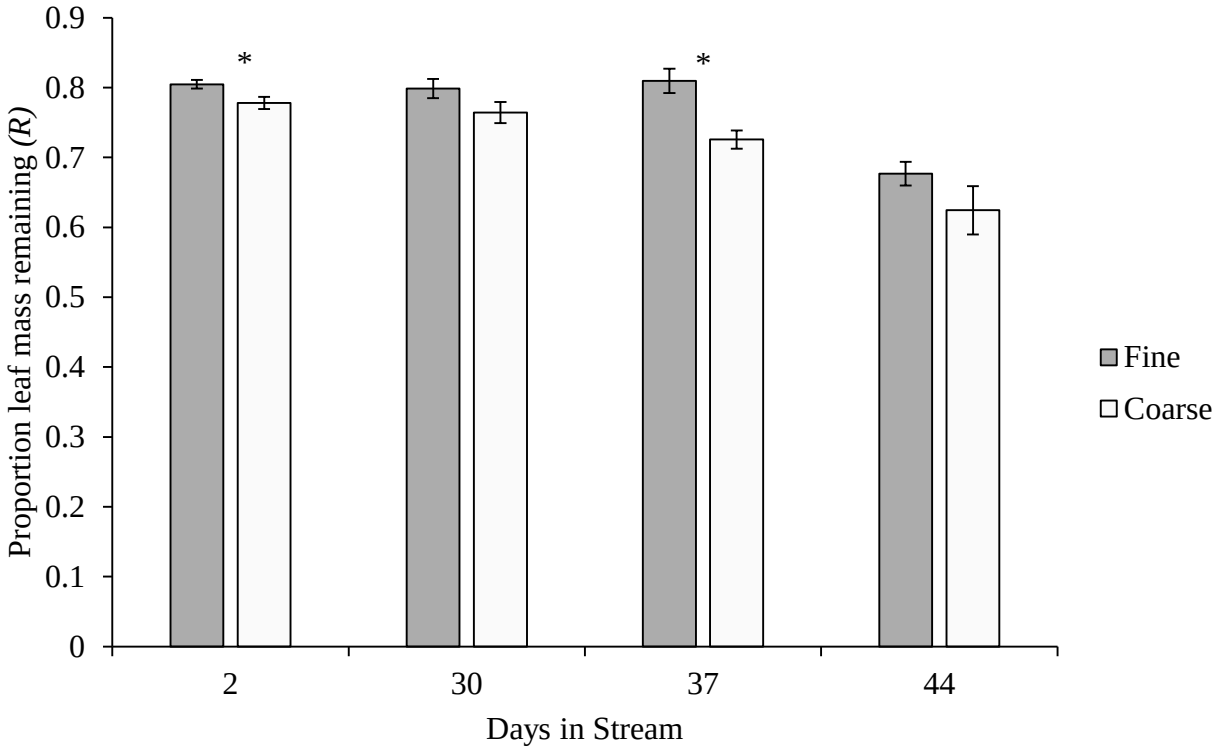

Figure 2: Leaf mass remaining (*R*) in fine and coarse mesh bags from pooled averages of open canopy and closed canopy reaches. Asterisk indicate significant differences in mesh type.

The invertebrate community consisted of seven broad groups: the insect orders Diptera, Coleoptera, Ephemeroptera, Plecoptera and Trichoptera, Class Gastropoda (entirely snail species), and "other". The latter included anything that was identified less than 5 times over the study period and included various arthropods, mites, nematodes and worms. These broad groups can be distinguished by feeding characterises as seen in Table 2 (Petersen & Cummins 1974, Chauvet et al. 1993, Tuchman & King 1993). Invertebrate counts were averaged for the six litter bags collected per mesh type and used to produce 100% stacked bar graphs (Figure 3). Diptera was the most abundant, occurring in all mesh types and on all collection dates, accounting for 62.7 to 97.1% of the invertebrate community. There was little change in proportion of Diptera in fine mesh bags (Figure 3A). In coarse mesh bags a larger proportion of shedder orders, such as Ephemeroptera and Plecoptera, appears after 37 days (Figure 3B), which could account for the greater decrease in *R* in coarse mesh bags (Figure 2). Total invertebrate abundance was also consistently higher in coarse mesh bags, although there was a significant difference only after 44 days in stream (Table 3).

Table 2: Function feeding groups of broad invertebrate groups

Invertebrate Grouping	Feeding Characterises
Diptera	Fine particle feeders, collector gatherers, scrapers
Coleoptera	Fine particle feeders, shredders
Ephemeroptera	Fine particle feeders, shredders
Plecoptera	Large particle feeders, shredders, predators
Trichoptera	Large particle feeders, shredders
Gastropoda	Scrapers

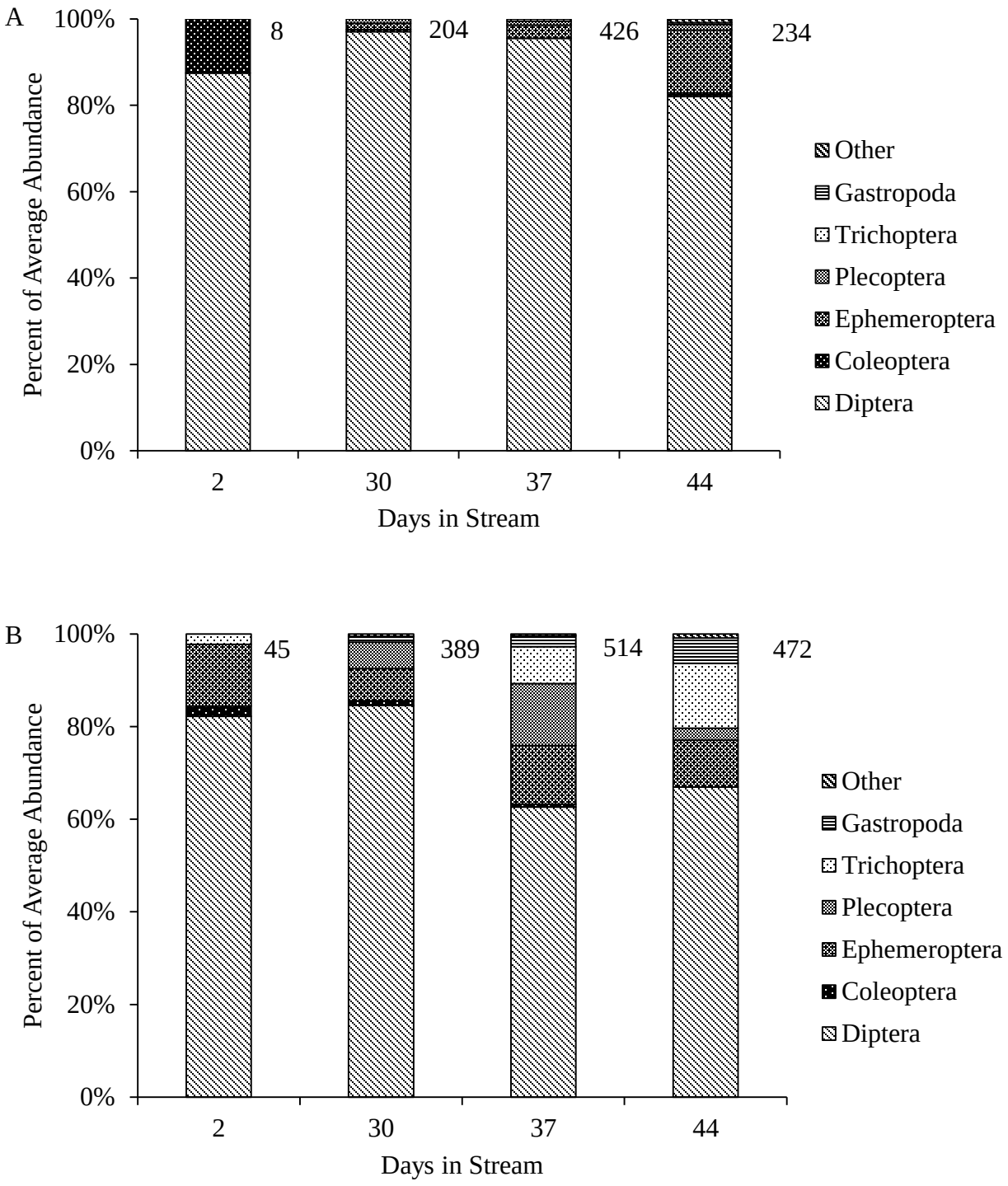

Figure 3: Stacked graphs of pooled average group abundance in fine mesh bags (A) and coarse mesh bags (B) after 2, 30, 37 and 44 days in stream. Total invertebrate abundances are shown to the right of each bar.

Table 3: Summary statistics of independent sample, equal variance t-tests conducted at a 95% confidence interval testing total invertebrate abundance between fine and coarse mesh bags from pooled open and closed canopy reaches.

Days in Stream	t	df	p
2	-2.052	10	0.067
30	-2.09	10	0.063
37	-0.942	10	0.369
44	-3.405	10	0.007

DISCUSSION

Open canopy and closed canopy reaches showed no significant difference in leaf mass loss which is contradictory to the findings of other studies. Leaf decomposition is normally higher in open canopy reaches due to an increase in microbial enzyme production and invertebrate feeding (Lagrue et al. 2011). Identification of the stream reaches as open and closed canopy sites was based on logging practices and subsequently assessed by surveying of riparian vegetation. The observed lack of difference in leaf breakdown could be due to physical differences in location of riparian vegetation: the open canopy reach had early successional shrubs (eg. alder) very close to the stream, which could have resulted in smaller difference in the amount of light reaching the stream compared to the closed canopy site. Alternatively, the lack of difference could be due to the microbial community response to light intensity. Microbial activity may not always increase in open canopy areas (Lagrue et al. 2011). The microbial community was unable to be analyzed in this study, so this cannot be confirmed. As data on light intensity was retrieved from only one logger, no comparison between reaches could be made. Future research should focus on a more secure placement of loggers to be able to obtain data on light intensity and preferably use this data to select study reaches, to ensure that they differ enough in light levels to be able to expect a biotic response.

Leaf mass remaining (*R*) values in fine mesh bags were consistently higher than those of coarse mesh bags which was expected as this pattern has been seen in studies such as Bergfur et al. (2007), Muto et al. (2011) and Köning et al. (2014). In these studies the differences between mesh types was shown by breakdown rates and percentage leaf mass loss. Differences in *R* were significant after 2 and 37 days in stream rather than on all days and this could be due to the small sample size making potential differences difficult to detect. A slight increase in *R* can be seen in

fine mesh bags after 37 days in the stream which was not expected but may be account for by differences in the interaction between the fungal and invertebrate communities in fine and coarse mesh bags. At that point in the breakdown process (30-37 days), fungal biomass would be expected to increase (Sridhar & Bärlocher 2000, Gessner et al. 2010). In the fine mesh bags, this may have been sufficient to offset any leaf mass losses and the increase in biomass was not consumed by the invertebrate community. In coarse mesh bags, fungal biomass would be rapidly consumed by the larger number of different feeding groups of invertebrates.

Leaf mass loss is usually characterized by large decreases in the first collection dates with decreases in loss occurring over time. After 2 days in stream leaching normally results in a 15% loss of leaf material and for birch leaves values have been shown to be 18% (Petersen & Cummins 1974, Haapala et al. 2001, Muto et al. 2011). The mass loss over the initial 2 day period observed in this study (~20% in fine and ~22% in coarse mesh bags) was in the expected range, and may have been slightly higher than previously published values due to the season in which the study was conducted, as studies are normally conducted in the fall when water temperatures may be lower, resulting in slower leaching rates.

Differences in invertebrate abundance between mesh sizes was significant only after 44 days while significant differences in *R* were seen at 37 days which could be due to lag effects between *R* and invertebrate abundance. Bergfur et al. (2007) found the same results with no relation between invertebrate and leaf breakdown at 34 days but after 55 days a relation was found. Bergfur et al. (2007) caution conclusions made about the relationship between invertebrates and leaf breakdown depend on the length of time leaves are in a stream.

The current study was conducted as a pilot to develop protocols, it was somewhat limited in scope. The length of the study was restricted by time and available resources. Due to conflicts with student academic schedules and difficulties of site access due to poor road conditions in fall and winter, the study took place in summer. Similar studies in the area should be conducted during autumn months, as well as for longer than 44 days as this may show results varying from the ones present. Carrying on the study for a longer period would allow for complete colonization by microbial and invertebrate communities (Sridhar & Bärlocher 2000) and may revel different relationships between leaf breakdown and measures of invertebrate communities (Bergfur et al. 2007). Placing leaf litter bags in a stream during autumn may show smaller values of *R* as this is the growth period of invertebrates and larger invertebrates are able to processes larger amounts of material (Petersen & Cummins 1974, Haapala et al. 2001, Muto et al. 2011). This study was conducted in two reaches of one stream rather than multiple streams, and with only one leaf species, due to time and resource constraints. The study of many streams within the Pynn's Brook area would provide a better understanding of leaf breakdown of the whole area. The use of multiple leaf species within a leaf bag or multiple bags with different species should be considered for future research. Mixtures of leaf species with varying quality may affect breakdown rates in at least two ways: priming/conditioning effects and feeding continuums. In priming, the presence of high quality material speeds the breakdown of more resistant low quality material (Gessner et al. 2010, Danger et al. 2013). Diversity of leaf species allows for a continuum in leaf species use to take place, which can support larger populations of detritus-feeders over long periods. The highest quality material is consumed first, leaving more resistant material for later consumption, when resources become more scarce (Petersen & Cummins 1974, Haapala et al. 2001, Kominoski et al. 2009).

CONCLUSIONS

Expected differences in leaf mass remaining (*R*) between open and closed canopy reaches were not observed, perhaps because light levels did not differ as expected based on logging history. Therefore, the hypothesis that leaf decomposition will be higher in the open canopy area cannot be accepted. The exclusion of large shredder invertebrates, accomplished by mesh size, resulted in significant reductions in *R* after 2 and 37 days in stream. The difference in *R* was small at 2 days and might not necessarily be a biologically meaningful difference. At 37 days, the difference was larger and could be attributed to the invertebrate community. Results from t-tests showed a significant difference in total invertebrate abundance only after 44 days. Therefore, it is unlikely that the differences in *R* seen were determined only by total invertebrate abundance. Although significant differences in invertebrate abundance and *R* between mesh types did not occur on the same dates, it is likely that a combination of invertebrate abundance and the larger proportion of shredding invertebrates seen in coarse mesh bags accounts for the observed differences in *R*. Thus, the proposed hypothesis, if invertebrate abundance is higher in coarse mesh bags then leaf mass loss will be higher can be at least partially accepted.

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