The Decomposition of Leaf Litter in Litter Traps:

Implications on Forest Biogeochemical Cycling

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

This research evaluates the decomposition of leaf litter while in litter traps. More specifically this study asks, 'Does sugar maple (*Acer saccharum* Marsh.), American basswood (*Tilia Americana* L.) and American beech (*Fagus grandifolia* Ehrh.) leaf litter collected bi-weekly from litter traps undergo a loss of dry mass and nutrient content (C, N, P, K, Ca and Mg) in comparison to freshly abscised leaf litter?'The objective of the initial experiment was to determine if sugar maple, basswood and beech leaf litter collecting in litter traps, while exposed to in-situ conditions, experienced decomposition. Results indicated that sugar maple, basswood and beech leaf litter experienced early stages of decomposition and identified precipitation, freezing temperatures and microbial activity as possible mechanisms for the observed decomposition. It was found that the dry weight of sugar maple and basswood differed significantly ($p < 0.05$ and $p < 0.10$, respectively) post- 14-day experiment period as compared to the initial dry weight. Consequently, three experiments were completed to examine the aforementioned variables. Conclusions were based on measured changes in the mass and nutrient (C, N, P, K, Ca and Mg) content of freshly abscised sugar maple, basswood and beech leaf litter under ex-situ conditions. It was found that the dry weight sugar maple and basswood leaf litter exposed to 30 mm, 60 mm and 100 mm of precipitation differed significantly ($p < 0.05$) as compared to freshly abscised leaf litter. In general, this research affirmed that precipitation and freezing temperature contribute to a change in mass and nutrient content of leaf litter collecting in litter traps. Furthermore, through measurable production of CO_2 and Community Level Physiological Profiling it was determined that microbes are present and active on the leaf surface and contribute to the decomposition of leaf litter in litter traps.

ACKNOWLEDGEMENTS

Table of Contents

List of Figures

List of Tables

Chapter 1

General Introduction and Literature Review

1.1 Problem Statement

Leaf litter is an intrinsic component of the ecological integrity of a forested ecosystem. This is evident through the many processes within a forest ecosystem that are influenced by the quality and quantity of leaf litter. Specifically, studies on leaf litter evaluate the leaf litter quantity and nutrient content to determine energy fluxes and productivity (Meier et al., 2006). This is largely because leaf-litter, and its subsequent decomposition, strongly influences primary production and regulates energy flow and nutrient cycling in forest ecosystems (Waring and Schlesinger, 1985). Thus, leaf litter is a major participant in the transfer of energy and nutrients in a forest ecosystem (Guo and Sims, 1999; Villela and Proctor, 1999).

Leaf litter is often collected by means of open traps, referred to as a litter trap, set-up under the forest canopy and above the forest floor. The sampling period and frequency of leaf litter collection from the litter traps differs among studies. The frequency of leaf litter collection is important to ensure quality and quantity, otherwise nutrients and soluble compounds may be leached out by rain and wet litter can begin to decompose and lose mass (Berg and Laskowski, 2006). In many studies leaf litter is collected from the traps on a biweekly or monthly basis during peak leaf litter production, normally September to November in northern temperate systems (Gosz et al., 1972, Ukonmaanaho and Starr, 2001). Some studies have identified that mass and nutrients are lost from the collected leaf litter, however, the relationship between the quantity lost and the processes involved are unknown (Oelbermann, 1999; Ukonmaanaho and Starr, 2001). Therefore, this unaccounted loss of nutrients, via decomposition, could potentially alter the current understanding of the role of leaf litter in biogeochemical cycling in forested ecosystems.

To date, it is believed that no study has quantified the loss of mass and nutrients as a result of processes of decomposition of leaves collecting in litter traps as part of leaf litter and nutrient flux investigations. The purpose of this study is to address the aforementioned gap in the

literature. To first address this problem a literature review was conducted. The following literature review section is intended to provide greater context and understanding of the theories and concepts governing this research.

1.2 Biogeochemistry in Forested Ecosystems

The study of energy and nutrient distribution, and the dynamics of chemical elements within an ecosystem are referred to as biogeochemistry (Kimmins, 2004). Nutrient cycling in a forested ecosystem largely determines the system's characteristics. Decades of research found the abundance, distribution and productivity of organisms within an ecosystem proportional to the availability and transfer of energy (Bray and Gorham, 1964). In general, a forest is an open ecosystem where nutrient and energy are influenced by inputs gained from various interacting sources, outputs lost to different sinks, and an array of internal cycling (Vitousek and Reiners, 1975). Nutrient cycling within a forest ecosystem is displayed in Figure 1.1. There are three basic components of biogeochemistry: geochemical, biogeochemical, and biochemical (Switzer and Nelson, 1972; Kimmins, 2004).
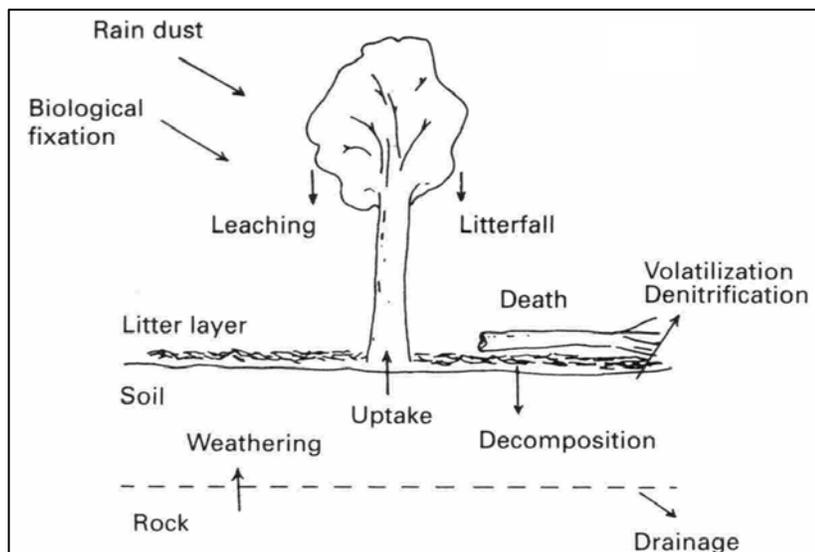
Figure 1.1: Biogeochemical cycle adopted from (Attiwill and Adams, 1993)

1.2.1 Geochemical Cycle

The geochemical cycle is the exchange of, or the inputs and outputs of, energy and nutrients between ecosystems (Switzer and Nelson, 1972; Kimmins, 2004). This involves, but is not limited to, inputs from rain, dust, weathering of rocks and biological fixation (e.g. carbon and nitrogen fixation), and outputs (denudation: transport of nutrients out of an ecosystem) to stream water, ground water and the formation of gas, as in denitrification (Attiwill and Adams, 1993; Kimmins, 2004). The input of nutrients from the weathering of parent material significantly contributes to mineral based nutrient reserves in forest ecosystems (Morris, 2000; Balogh-Brunstad et al., 2008). For example, the supply of phosphorus (P) and base cations (Ca, Mg, K and Na) from parent material is sufficient to ensure adequate plant growth (Morris, 2000). Biota and soils act as mediating agents in the weathering of parent material and subsequently as storage for the nutrients (Balogh-Brunstad et al., 2008).

The nutrient retention hypothesis predicts the biotic control on the biogeochemical cycle, specifically the biotic demand on nutrient input and output (Vitousek and Reiners, 1975; Gorham et al., 1979; Hedin et al., 1995; Balogh-Brunstad et al., 2008). This predicts that rapidly growing mid-successional forests, when forest productivity is high, significantly retain nutrients while early and old growth forests do not (input approximately equals output) (Vitousek and Reiners, 1975; Gorham et al., 1979; Cole and Rapp, 1981; Hedin et al., 1995; Balogh-Brunstad et al., 2008). For instance, Vitousek and Reiners (1975) explain that in an early successional state the input of nutrients from rainfall will be lost, in this case to hydrologic outputs, at a rate similar to that introduced due tothe the systems inability to conserve nutrients. As the system matures, compartments like biomass and soil organic matter are more apt to take-up and store elements resulting in higher inputs than outputs (Vitousek and Reiners, 1975). Biomass growth will eventually reach a steady state, net ecosystem growth will approach zero, and inputs again will be approximately equal to outputs (Vitousek and Reiners, 1975).

1.2.2 Biogeochemical Cycle

The biogeochemical cycle is the exchange of chemicals within an ecosystem (Kimmins, 2004). For example, nutrients are transferred and cycled from plant to soil (Attiwill and Adams, 1993). Also commonly referred to as uptake and return, the biogeochemical cycle is represented by the four major processes (Attiwill and Adams, 1993):

1. Nutrient uptake by plants from the soil for growth
2. Retention of nutrients within plants
3. Return of nutrients through leaf decay and leaching, and root turnover
4. And eventually the decomposition of the dead plant material (detritus)

The uptake and the retention of nutrients vary considerably depending upon the species, stand age, and site characteristics (Attiwill and Adams, 1993; Morris, 2000). The return of nutrients depends on the quantity and process of decomposition and is influenced by temperature, moisture, pH, and litter quality (nutrient chemistry) (Hobbie and Vitousek, 2000).The biogeochemical cycle is a process of positive feedback (Ehrenfeld et al., 2005). That is, plants can influence change in the composition and activity of soil biota, and the physical and chemical properties and the rates of ecosystem processes. In this way, the process subsequently affects the living conditions of the environment (Ehrenfled et al., 2005).

1.2.3 Biochemical Cycle

The biochemical cycle, commonly referred to as the internal cycle, is the redistribution of chemicals within individual organisms, also commonly described as the internal transfer relationship (Kimmins, 2004; Attiwill and Adams, 1993; Switzer and Nelson, 1972). Specifically, the biochemical cycle is the redistribution of elements within a plant. The process of retranslocation is the movement of inorganic and organic foliar nutrients from senescing leaves to surviving tissue (Killingbeck, 1986; Nambiar and Fife, 1991; Aerts and Chapin, 2000). Retranslocation of mobile nutrients like nitrogen (N), phosphorus (P), potassium (K), sulfur (S), copper (Cu) and zinc (Zn) is important when soils nutrient levels are depleted (Nambiar and Fife, 1991). As a result, retranslocation is a significant contributor to tree growth (Nambiar and Fife, 1991). For example, as much as 80% of P and N, and much less of other nutrients, have been

found to retranslocate from the foliage nutrient pool (Hagen-Thorn et al., 2006). Although retranslocation is commonly thought to be a reactive process to leaf senescent (Switzer and Nelson, 1972; Killingbeck, 1986), it has been found to occur from young and actively growing leaves (Nambiar and Fife, 1991).

Leaves are subject to the majority of internal nutrient retranslocation (Nambiar and Fife, 1991). However, it has been found that other plant tissue, like branch and stem tissue, experience nutrient retranslocation (Nambiar and Fife, 1991). The degree to which nutrients circulate within a tree differs among species and fluctuates according to soil fertility, leaf nutrient status, annual precipitation and summer temperature (Hagen-Thorn et al., 2006; Berg and Laskowski, 2006). Nambiar and Fife (1991) attribute differences in nutrient retranslocation to source-sink relationships, seasonality in growth patterns and the longevity of leaf life. With many fluctuating variables biochemical cycling of nutrient can be widely different from year to year. Although this study focuses on the biogeochemical cycle, and specifically nutrient cycling as it relates to leaf litter, all three cycles, geochemical, biogeochemical and biochemical, are interrelated and greatly influence leaf nutrient condition.

1.3 The Role of Leaves in Biogeochemical Cycling

The release of nutrient from leaves is an integral component of nutrients recycling (Robert et al., 1996; Rutigliano et al., 1998). It is, in fact, the principal pathway for the return of nutrients to the soil (Miller et al., 1979; Kavvadias et al., 2001). Leaves are an essential link between primary producers and consumers. The recycling of nutrients from leaves is a result of retranslocation (discussed earlier), leaching of the canopy, commonly referred to as throughfall, and leaf litter (Dechesne et al., 2001).

1.3.1 Throughfall

The transfer of solutes from the canopy to the forest floor, by rainfall, provides an important pathway for nutrient recycling. It is important for the biogeochemical and hydrological cycles, and many ecological processes (Parker, 1983; Levia and Frost, 2003; Staelens et al., 2006). Incident precipitation, defined as rainfall that has yet to be intercepted by vegetation is chemically altered as it moves through the vegetation (Eaton et al., 1973). Nutrients are picked

5

up through the processes of throughfall and stemflow. Throughfall is the precipitation that runs off of the canopy (Eaton et al., 1973). Stemflow is the precipitation that runs down the boles of the trees (Eaton et al., 1973). These processes add nutrients directly to the nutrient pool as these substances are already in a form for up-take unlike leaf litter which requires decomposition (Eaton et al., 1973). In general, stemflow has been found to be more nutrient rich than throughfall. However throughfall intercepts a greater amount of precipitation (Waring and Schlesinger, 1985; Levia and Frost, 2006; Parker, 1983). Robson et al. (1994) reported stemflow nutrient concentrations as almost twice that of throughfall in a broadleaf forest. Typically throughfall represent 60-90% of the precipitation collected (Parker, 1983). Throughfall is said to account for 90% of the annual return of nutrients that are leached (Waring and Schlesinger, 1985).

The interception of rainfall varies spatially (Beier et al., 1993). The solute concentration is inversely proportional to the distance from the tree trunk (Beier et al., 1993). Hansen (1996) found a similar spatial distribution of intercepted rainfall and ion concentration. Hansen (1996) reported decrease in interception fluxes further down the canopy structure (greater quantities at higher reaches of the canopy) and an associated increase in ion concentrations, as a result of further canopy interception. This is partly influenced by increased dry deposition at the top of the canopy, as this part of the tree is most in contact with turbulent air, where then lower levels of the canopy will be exposed to enriched throughfall (Hansen, 1996).The chemistry of the throughfall is altered by dry-deposited elements on the plant surface, the leaching of nutrients, and absorption of ions from rain (Eaton et al., 1973; Potter et al., 1991; Beier et al., 1993). More specifically, the nutrient content of the throughfall may be altered as elements within the incident rainfall are retained by the leaf canopy, either absorbed by the leaf or deposited as salts on the leaf surface. Nutrients may also be taken-up by microflora (Lovett et al., 1996; Eaton et al., 1973).

The extent of leaching can be attributed to characteristics associated with the environment and those associated with the plant. The concentration of ions in throughfall is related to the amount and intensity of a precipitation event (Pryon and Barthelmie, 2005; Eaton et al., 1973). It has been found that the proportion of nutrients leached and washed from the canopy is inversely

6

related to the magnitude of the storm (Robson et al., 1994). The majority of chemical alteration of throughfall occurs in the initial phase of a precipitation event (Levia and Frost, 2006). Hansen et al. (1994) found that in general the majority of ion (Na^+, Cl^-, Mg^{2+}, SO_4^{2-}) concentrations were highest at the beginning of the rain event and the concentration would continue to decrease until reaching a plateau. Throughfall ion concentrations also increase with longer durations of dry periods between precipitation events (Tobon et al., 2004).

Throughfall yield is related to canopy morphology, stand structure and leaf characteristics, such as leaf shape and orientation (Levia and Frost, 2006). For example, Staelens et al. (2006) found that the architecture of the canopy leaves and branches significantly influences spatial distribution of throughfall deposition. Staelens et al. (2006) also found a strong positive correlation in canopy cover to the deposition of ions (with the exception of H^+). The morphology of the canopy structure, namely the branch inclination angle and geometry influences the amount of incident rainfall that is intercepted as throughfall and stemflow (Pryon and Barthelmie, 2005; Herwitz, 1987). This results in intra-species differences (Pryon and Barthelmie, 2005; Herwitz, 1987). The amount of rainfall and the length of interaction with leaves, dependent on such factors as texture, cuticle thickness, petiole orientation and where nutrients are held within the biological material, affect the extent of chemical exchange (Levia and Frost, 2006; Eaton et al., 1973). Lastly, there is a significant relationship between the health of a forest, defined as one that maintains and sustains desirable ecosystem functions and processes (Natural Resources Canada, 2008), and the leaching of nutrient. Increased foliar leaching of base cations and organic compounds is found to be associated with the decline of forest health, specifically related to plant tissue damage (Balestrini and Tagliaferri, 2001). This also relates to the physiological condition of the plant tissue. Tukey (1970) stated that injured and senescing leaves are more susceptible to leaching nutrients then younger and intact leaves.

1.3.2 Leaf Litter

Leaf litter studies in forest stands have been conducted for over a century (Ebermayer, 1876; Zhou et al., 2006). The collection of leaf litter has been executed for a variety of reasons and in a variety of forest stands. Earlier studies focused on the amount, composition and

distribution of leaf litter (Zhou et al., 2006). More recently, leaf litter studies focus on the ecological role of leaf litter in nutrient cycling, and possible interactions with biotic and non-biotic variables (Zhou et al., 2006). In 1876, Ebermayer conducted the first major study primarily concerned with leaf litter in response to visible evidence of forest health deterioration. Ebermayer (1876) was interested in the effects of continued removal of leaf litter from the forest floor. Traditionally, leaf litter was removed from the forest floor and used as animal bedding in barns over the winter (Attiwill and Adams, 1993). Come spring, the litter, enriched with animal droppings would be spread over farm fields, thus removing significant nutrients from the forest ecosystem (Attiwill and Adams, 1993). Ebermayer's (1876) findings now form the basic foundation for leaf litter studies.

Leaf litter is essential to the ecological sustainability of a forest. The amount and nutrient composition of leaf litter has long been identified as important to energy and nutrient cycling. Many processes within the forest ecosystem are dependent on the quality and quantity leaf litter. For example, leaf litter has been studied to evaluate the nutrient content to determine energy fluxes and productivity (Bray and Gorham, 1964; Meier et al., 2006). This is largely because leaf litter, and subsequently decomposition, strongly influence primary production and regulate energy flow and nutrient cycling in forest ecosystems (Waring and Schlesinger, 1985). Along with providing nutrients for tree, leaf litter also provides energy and a living environment for the soil fauna and microorganisms (Guo and Sims, 1999). As well, the decomposition of leaf litter is necessary for soil fertility and the formation of organic matter (Guo and Sims, 1999), and the rate of decomposition regulates productivity (Swift et al., 1979). Thus, leaf litter is a major participant in the transfer of energy and nutrients in a forest ecosystem (Guo and Sims, 1999; Villela and Proctor, 1999).

As stated, the input of nutrients to the soil is the result of leaf litter, throughfall and stemflow (Pedersen and Bille-Hansen, 1999). However, of these three pathways leaf litter is the most important source of nutrient flux, providing a nutrient flux greater than that combined in throughfall and stemflow (Miller et al., 1979; Stevens et al., 1989; Ukonmaanaho and Starr, 1999). On average, leaf litter contributes 70% of the total aboveground litterfall (Meentemeyer et al., 1982). Once on the forest floor, leaf litter provides important ecological functions and

influences the internal nutrient composition of trees (Flower – Ellis and Olsson, 1978). Leaf litter can be used as an indicator of forest conditions as it is an integrated response of biological heredity of the trees and the influence of environmental fluctuations (Pedersen and Bille-Hansen, 1999). Climatic change or disturbance, as well as change in other environmental factors, may impinge leaf litter patterns (Pedersen and Bille-Hansen, 1999). Foliage, or the loss of foliage, was measured as an indicator of the ecological integrity of a forest (Pedersen and Bille-Hansen, 1999). Pedersen and Bille-Hansen (1999) compared the amount of leaf litter and its element concentrations and fluxes between even-aged stands to evaluate leaf litter as an indicator of forest health. Pedersen and Bille-Hansen (1999) determined that leaf litter can be used as an indicator of forest health reflecting major disturbances from the surrounding environment. Zhou et al., 2006 also stated that the quality and quantity of leaf-litter produced in a forested ecosystem is influenced by environmental factors, such as acid rain.

The decomposition of leaf litter is fundamental to the healthy operation of a forest ecosystem (Graca et al., 2005). Decomposition is the breakdown of dead organic material by organisms, such as bacteria and fungi, and physical forces, such as freeze-thaw and leaching (Leuschner, 2005). Essentially, the decomposition of leaf litter can be divided into three phases, a fast loss in mass from the leaching of the soluble components, subsequent colonization by microorganisms, also referred to as conditioning, and degradation by invertebrate feeding and physical abrasion, also referred to as breakdown (Boulton and Boon, 1991). Decomposition allows for the breakdown of complex energy rich molecules, including carbohydrates, proteins and lipids, into carbon dioxide, water and inorganic nutrients (Leuschner, 2005). Mineralization is the process in which decomposition releases inorganic compounds (CO_2, H_20, NH_4^+ and Ca_{+2}) (Waring and Schlesinger, 1985; Leuschner, 2005). Thus, decomposition and mineralization complete the nutrient cycle and are vital to forest productivity and the global carbon budget (Prescott, 2005). On a regional scale, climate is assumed to have the most significant effect on litter decomposition rates, whereas on a local scale litter quality will dominate the decomposition rate (Berg and Laskowski, 2006). In a global context, 71% of the differences in the rate of mass loss of leaf litter is accounted for by annual precipitation and evapotranspiration (Dyer et al., 1990). Freshly fallen litter contains high levels of simple compounds, such as simple sugars,

9

lower fatty acids and proteins (Berg and Laskowski, 2006). Initial decomposition causes a rapid decrease of these molecules as they are easily taken up by microorganisms and leached (Berg and Laskowski, 2006). Leaching may account for up to a 30% loss of the water soluble compounds (Berg and Laskowski, 2006).

1.4 Litter Collection Methods: The Litter Trap

To study leaf litter, a device referred to as the litter trap is used to collect samples. Generally, the litter-trap is constructed to a known surface area and used to catch naturally abscised material from the overhanging canopy, which is subsequently collected and analyzed (Morris, 2000). Studies on the litter trap have primarily focused on size, shape and distribution. As part of the International Biological Program (IBP), Newbould (1967) standardized the litter trap design. Newbould (1967) specified the trap to have an opening of 0.5 m^2 and to be conically shaped. This was meant to allow comparison of data between different communities for global wide usage (Burquez et al., 1999). Despite the simplistic design, it has not been widely adopted, likely because of the variety of environments and requirements for which the litter trap is used (Burquez et al., 1999). The litter trap has been built and modified to meet a variety of circumstances. Morrison (1991) studied four trap designs with varying height and surface area in a Canadian old growth sugar maple forest. Although no trap type displayed a distinct advantage, analysis of variance showed that height and surface area significantly affected the amount of litter that was trapped (Morrison, 1991). One significant conclusion Morrison (1991) found was that traps should not be placed in microsites that support the accumulation of windblown leaves. As well, slight modifications to the basic design can be made to reduce loss of leaf litter. For example, Pedersen and Bille-Hansen (1999) increased the brim height to reduce the common onset of air turbulence from lifting leaf litter from the trap. Burquez (1999) examined the litter trap design for desert-like ecosystems. This required a trap located much closer to the ground as the ecosystem is largely dominated by shrub like species (Burquez, 1999). Chapman (1983) proposed necessary guidelines for proper litter-trap utilization. These include: the trap must intercept litterfall before reaching the ground (minimizing aerodynamic disturbance), litter must be held within the trap, litter on the soil surface cannot be able to enter the trap, water should be

10

able to drain without causing loss of litter, and lastly the size and number of traps much be adequate to provide an appropriate degree of accuracy (Chapman, 1983).

It is agreed that litter traps should be placed randomly over the plot (Berg and Laskowski, 2006). As well, the mesh size of the catchment netting should vary depending on the foliage being collected (Berg and Laskowski, 2006). The sampling periods and frequency of collection differ among studies. The frequency of leaf litter collection is important to ensure quality and quantity. This is because nutrients and soluble compounds may be leached out by rain and wet litter can begin to decompose and lose mass (Berg and Laskowski, 2006). It is best to collect litter that is shed naturally as litter collected off of the tree may have a different chemical composition (Berg and Laskowski, 2006).

1.5 Nutrient Characteristics of Leaf litter

The amount of elements leached from leaved varies according to season and the type of tree (Waring and Schlesinger, 1985). Leaching rates increase as leaves senesce, but can differ between leaf types depending on nutrient concentration, surface-area-to-volume ratio, surface texture and leaf age (Waring and Schlesinger, 1985).

Throughout the growing season, nutrients leaching from the canopy follows K>P>N>Ca (Waring and Schlesinger, 1985). Similarly, Eaton et al. (1973) stated that the mobility and leachability of elements from the canopy during summer months is greatest in Na and sequentially lower in S>K>Mg>Ca>N>P. Eaton et al. (1973) found that the leaching of Ca and Mg remained constant throughout June, July and August and maximized in September, and then declined just before senescence. The leaching of N was variable throughout the summer and then also reached a maximum in September followed by a decline before senescing (Eaton et al., 1973). The actual leaf content of N, P and K is kept at a relatively high level throughout the growing season and is largely removed from leaves in the fall, mostly through retranslocation. Total organic matter concentrations were found to be relatively low throughout the summer, but increased to highest measured levels before senescence (Eaton et al., 1973). Furthermore, concentrations of ammonium (NH_4^+), nitrate (NO_3^-) and hydrogen ions (H^+) in throughfall have been found to be taken up by plants throughout the growing season (Hansen, 1996; Tukey,

1970). In general, soluble forms of N have been shown to be absorbed by leaves from rainfall (Waring and Schlesinger, 1985).

As previously stated, nutrients leaching from leaves depend on a number of variables. Physical characteristics, including leaves with a smooth and waxy surface are more resistant to becoming wet as readily as other leaves and are less likely to leach nutrients (Tukey, 1970). With that said, almost all leaf constituents leach, including macro and micro-elements, organic and inorganic substances (free sugars, pectic substances and sugar alcohols), and amino and organic acids (Tukey, 1970). For the most part organic substances, mainly carbohydrates, represent the considerable component of leached materials (Tukey, 1970), although inorganic elements are found to be more mobile (Eaton et al., 1973; Tukey, 1970). Eaton et al. (1973) suggest this is the case because inorganic elements are readily exchangeable as they originate from cell sap. Organic elements are associated with plant tissue and storage and, therefore, rely more on the processes of decomposition in recycling (Eaton et al., 1973). The decomposition of litter releases inorganic elements, including Ca, K and Mg, and organic compounds, such as sugars and proteins (Ibrahima et al., 1995).

The release of nutrients from decomposing litter is not a consistent characteristic as it is influenced by a number of variables. Litter deficient in a nutrient is likely to be immobilized in the initial stages of decomposition, while nutrients in abundance would readily be released (Swift et al., 1979; Laskowski et al., 1995). Laskowski et al. (1995) summarized a general pattern for the release of nutrients from decomposing litter in temperate forest ecosystems. Laskowski et al. (1995) stated that N and P exhibit a similar pattern in decomposing litter. Nitrogen and P usually increase in the initial stages of decomposition and then are released (Laskowski et al., 1995). However, it has been observed that N and P sometimes decrease slightly before beginning to increase (Laskowski et al., 1995). Inorganic elements like Ca, K and Mg are usually observed to decrease in absolute amounts (Laskowski et al., 1995). This may mean that the concentration of these elements continues to be constant, as has been most notably seen in Ca, or the concentrations may decrease, as is most frequently observed in K and Mg (Laskowski et al., 1995). Laskowski et al. (1995) associated the increase of nutrients, whether it be N, P or K, to

microbial immobilization (if it is a relative increase) or inputs from an external source (if it is absolute).

Swift et al. (1979) suggest that the rate of nutrient turnover follows, K>Ca>Mg>P>N. In agreement, Attiwill (1967) suggested the order of the mobility of nutrients from decomposing litter is K>Ca>Mg>P. This essentially takes into account the readily mobilization of K and Mg from plant litter, and there low residence time in decomposing litter (Tukey, 1970; Swift et al., 1979). Nitrogen and P, on the other hand, are measurable in decomposing litter for much longer (Swift et al., 1979). Tukey (1970) reported highest levels of leaching of inorganic nutrients, like K, Ca, Mg, Mn, as opposed to N and P. This said, physical condition and the elemental concentration of the leaves is by far the greatest determining factor in the rate of leaching (Eaton et al., 1973). Leaf litter generally has lower concentrations of N, P and soluble carbohydrates, due to retranslocation, then living leaf tissue. As well, in most cases leaf litter has higher concentrations of Ca, tannin and leaf structural material, like lignin (Waring and Schlesinger, 1985). Microbial growth frequently immobilizes N and P that may have otherwise been lost through processes of decomposition. Elements like K and Mg are lost relatively quickly from litter as they are not usually limiting for microbial growth (Waring and Schlesinger, 1985). Thus they are released as soluble ions to the soil (Waring and Schlesinger, 1985).

1.4.1 Calcium (Ca)

The loss of calcium (Ca) from decomposing litter is slow due to its importance as a structural component. Its loss is correlated with the breakdown of lignin (Attiwill, 1967). Calcium is not retranslocated because of its position within the leaf, thus rendering it immobile (Chapin, 1980; Cole and Rapp, 1981; Ostman and Weaver, 1981; Likens et al., 1998). For forested ecosystems, the recycling of Ca is largely dependent on the uptake of nutrients from the decomposition of litter, as opposed to leaching (Gosz et al., 1973; Likens et al., 1998).

The availability of Ca in forests is a combination of atmospheric supply, cation exchange, mineral weathering, decomposition of soil organic matter (SOM) and from the leaching and sequester of vegetation (Likens et al., 1998). Waring and Schlesinger (1985) reported that Ca increases in leaves before abscission. Similarly, Dechesne et al. (2001) measured the

13

concentration of Ca over an entire season (in sugar maple and beech) and determined that Ca concentrations are low in the early spring and increase throughout the season until senescence. Lastly, litter is the most prevalent source of Ca in a forest ecosystem, approximately 88% of the Ca cycled (Ostman and Weaver, 1982).

1.4.2 Potassium (K)

As opposed to Ca, K is readily leached from leaf litter because it is highly soluble (Likens et al., 1994; Likens et al., 1998) and concentrated in cells present close to the leaf surface (Waring and Schlesinger, 1985). For the same reasons, throughfall is considered an important source of K as it is easily leached from the canopy (Waring and Schlesinger, 1985). Joergensen and Meyer (1990) stated that K is electrostatically bonded to cell membranes, and therefore readily leached as membranes are destroyed. The retranslocation properties of K are debated. While Cole and Rapp (1981) stated that very little K is reabsorbed. Ostman and Weaver (1982) reported that approximately 63% of K was retraslocated. As well, Waring and Schlesinger (1985) stated that K is readily reabsorbed before abscission, along with N and P. The differences found may relate to the earlier discussed circumstances which affect the cycling of nutrients in this case the efficiency of recycling required (nutrient use efficiency). Many studies have observed the rapid decrease in K in the initial stage of decomposition (Rutigliano et al., 1998; Laskowski and Berg, 1993). Potassium concentrations, specifically measured in beech and sugar maple, are highest in May and from there decrease over the season, reaching lowest measured levels at senescence (Dechesne et al., 2001).

1.4.3 Magnesium (Mg)

Cole and Rapp (1981) report that, similar to Ca, Mg is not retranslocated and is actually available in abundance. Therefore, no nutrient cycling efficiency is required. As opposed to Ca, which is structurally bonded, Mg is generally more soluble (Joergensen and Meyer, 1990). Mg is present in plant tissue in chlorophyll, enzymes and in salts (Joergensen and Meyer, 1990). Although, Gosz et al. (1973) stated that Mg acts similar to K in that it rapidly leaches from litter during the initial stages of decomposition, Staff and Berg (1982) reported conflicting results. Staff and Berg (1982), as well as Laskowski et al. (1995) found that Mg concentrations remained

14

constant, thus implying a direct relationship between Mg release and litter decomposition. Similarly, Joergensen and Meyer (1990) found that beech leaf litter released Mg and Ca at the same rate as C was lost.

1.4.4 Phosphorus (P)

Phosphorus is one of the most tightly cycled major plant nutrients and is an essential plant macronutrient (Wood et al., 1984; Yanai, 1992; Fiorentino et al., 2003). Usually more than half of the P in deciduous leaves is retranslocated back to the tress before leaf abscission (Chapin, 1980). Phosphorus (P) is found to initially leach from leaf litter and then is lost through microbial decomposition in later stages of decomposition (Rutiglaine et al., 1998). In some cases the concentration of P in decomposing litter has been reported to decrease, while others report that P remains constant (or a relative increases) (Morre et al., 2006). This is a characteristic of the leaf litter quality and the site, namely whether P is limited (Moore et al., 2006). Rutigliane et al. (1998) suggested that the increase in P was the result of microorganism immobilization as it was a limiting resource. This is also used to explain the increase in N.

1.4.5 Nitrogen (N)

In leaves, Nitrogen (N) is bound to C, for this reason the cycling of N is dependent on the cycling of C or the release of N from C through mineralization (Cole and Rapp, 1981). Nitrogen can also become available through fixation, nitrification and denitrification (Cole and Rapp, 1981; Laskowski et al., 1995). For the most part N is the most frequently limited nutrient in forested ecosystems (Prescott, 2002).

Cole and Rapp (1981) report that N from atmospheric input is greater then is lost through leaching. More N is added by precipitation then is lost by leachate. Generally, the greatest transfer of N back to the plant is by retranslocation, an estimate of 78.5% (Cole and Rapp, 1981; Ostman and Weaver, 1982). T he remainder is returned back to the system through decomposition of leaf-litter (Cole and Rapp, 1981). During the decomposition of litter, N may be lost initially through leaching, followed by a longer period in which N increases, at least relative to C (Laskowski et al., 1995). The increase in N is associated with microbial fixation of

15

atmospheric N_2, inputs from external sources, like throughfall, and microbial immobilization (Laskowski et al., 1995).

1.4.6 Carbon (C)

The decomposition of litter is largely dependent on the chemical quality (Aerts, 1997). This is most often related to the ratio of carbon (C) to N, as well as the ratio of C to P (Taylor et al., 1989; Aerts, 1997). The C/N ratio, and other C/element ratios, are litter quality characteristics which modify the decomposition rate of organic substrates (Flanagan and Van Cleve, 1983). Chemical quality directly influences microbial activity and mineral release (Flanagan and Van Cleve, 1983). This has been found to be strongly correlated to the content of N. Nitrogen limitations largely limit microbial production (Taylor et al., 1989). Thus N is often immobilized in litter during early stages of decomposition (Taylor et al., 1989). In general, the rate of organic matter decomposition is inversely related to the C/N ratio and is directly related to the N and P concentration of the litter (Flanagan and Van Cleve, 1983). High C/N ratios result in slow decomposition rates (Melillo et al., 1982; Flanagan and Van Cleve, 1983; Aber at al., 1990). In N-limited conditions the C/N ratio will decrease without the loss of N until a 'critical' level is reached (Joergensen and Meyer, 1990).

Carbon to element ratios is used to relate the loss of an element to the loss of dry weight (Lousier and Parkinson, 1978). If the ratio increases, the release of the element is often due to leaching or mineralization processes (Lousier and Parkinson, 1978). If the ratio decreases, the element is accumulating through biological or physiochemical processes (Lousier and Parkinson, 1978). If the ratio remains constant then the release of the element is related the dry weight (Lousier and Parkinson, 1978; Staaf and Berg, 1981). Taylor et al. (1989) stated that the critical C/N ratios is 30:1, litter with C/N >30:1 initiate N immobilization, while litter <30:1 cause N mineralization. The critical ratio for C/P is more inconsistent (Taylor et al., 1989). Lousier and Parkinson (1978) reported that litter <230:1 initiates mineralization. The critical value represents the heterotrophic demand for the element and/or the value at which the element is released (Lousier and Parkinson, 1978).

1.4.7 Dissolved Organic Carbon (DOC)

Dissolved organic carbon is an important source of C to a forest ecosystem (Neff and Anser, 2001; Peichl et al., 2007). It is also important to soil formation processes (Neff and Asner, 2001). DOC in leachate from the forest floor consist of high-molecular-mass complexes of organic acids, often characterized as humic or prehumic substances, formed from incomplete decomposition and microbial modification (Currie et al., 1997; Cronan and Aiken, 1985).

Dissolved organic carbon is defined in this study as solutes that are able to pass through a filter with pore size of <0.70 µm (Michalzik et al., 2001). The leaching of DOC from litter is controlled by biological, chemical and physical processes (Michalzik et al., 2001), of which temperature and precipitation are known to have the greatest affect. This mostly due to their effect of microbial activity and leaching patterns (Godde et al., 1996). Godde et al. (1996) found that temperature increased the solubilization and mobilization of DOC. This was explained by Godde et al. (1996) as a microbial dependent relationship; DOC is released by the activity of microbes and the activity of microbes is increased in higher temperatures. As well, Godde et al. (1996) concluded that DOC leaching was affected by the frequency of treatment application. Total DOC leached was greater within litter exposed to regular treatments, but was less concentrated after each application, as opposed to less regular applications (Godde et al., 1996). Michalzik et al. (2001) found that DOC leaching for the forest floor litter is positively correlated to annual precipitation. The affect of precipitation pH on DOC leaching is variable (Michalzik et al., 2001), whereas litter quality (measured as C:N ratio) is positively related to the loss of DOC (Godde et al., 1996). Godde et al. (1996) found that higher C:N value were associated with greater loss of DOC. However, microbial organisms are unable to degrade DOC (Godde et al., 1996; Qualls and Haines, 1992). Although many studies have indicated that the release of DOC is related to microbial activity, in early stages of litter decay the release of organic matter (DOM in general) can leach irrelevant of microbial requirement for inorganic nutrients (Kalbitz et al., 2000). Whether or not litter C/N ratios affect DOC leaching is debatable. While Michel and Matzner (1999) and Michalzik and Matzner (1999) found that the flux of DOC was not related to litter C/N ratios, Godde et al. (1996) reported a positive relationship.

1.4.8 Dissolved Organic Nitrogen (DON) and Dissolved Inorganic Nitrogen (DIN)

In most cases the majority of total dissolved nitrogen is dissolved organic nitrogen (DON), and the majority of DON is amino N (Michalzik and Matzner, 1999). Dissolved organic nitrogen from leaf litter represents a significant flux of N into the soil (Michalzik and Matzner, 1999). The release of DON from litter is dependent on abiotic processes (Michalzik and Matzner, 1999). Dissolved organic nitrogen is defined as the difference between mineral N (NO_3^- and NH_4^+) and total dissolved N (organic and mineral N) (Michalzik and Matzner, 1999; Park et el. 2002). In this study DON is calculated as the difference between Total Kjehldahl Nitrogen (Organic N and NH_4^+) and NH_4^+.

Temperate and northern regions are most often characterized as N-limited (Vitousek and Howarth, 1991 from Neff). Dissolved organic nitrogen is more abundant than inorganic N in terrestrial ecosystems (Neff et al., 2003). Dissolved organic nitrogen enters a forest ecosystem through the interaction of precipitation with soil and vegetation (Neff et al., 2002). The largest flux of DON is found in throughfall and in the surface layers of soil solution. In contrast to dissolved inorganic N, the forest canopy is a source for DON (as well as DOC) (Michalzik et al., 2001). Fluxes of DON in throughfall are positively related to fluxes of NH_4^+ and NO_3^- (Michalizik et al., 2001). Unlike DON fluxes in throughfall, the litter layer DON fluxes are also positively correlated with annual precipitation (Michalzik et al., 2001). Mean annual precipitation is positively related to the annual flux of DON (as well as DOC) from the litter layer (forest floor) (Michalzik et al., 2001). However, Michalizik et al. (2001) concluded that it was indiscernible whether precipitation directly influenced the leaching of DON (and DOC) or whether precipitation enhanced optimal moisture conditions. The release of DON from litter has not been found to be related to the C/N ratio of plant material (Michel and Matzner, 1999), to levels of NH_4^+ and NO_3^- from leaf litter leachate, or to leaf litter (Michalizik et al., 2001). Furthermore, Michalizik et al. (2001) reported that DOC and DON fluxes and concentrations are highly correlated. Both respond similarly to precipitation (Michalizik et al., 2001). Dissolved organic nitrogen encompasses a number of compounds, ranging from simple compounds that can easily be taken up by plants and microbes to more complex compounds like polyphenols and tannins (Neff et al., 2003). Dissolved organic nitrogen that leaches from plant litter is highly

18

decomposable (Neff et al., 2003). Dissolved organic nitrogen is the main form of N to leach from the forest floor (Cortina et al., 1995).

1.6 Research Objectives

As previously mentioned, many studies have suggested that leaf litter is subjected to nutrient leaching while collecting in litter traps (Mulholland, 1981; Inverson et al., 1982; Benson and Pearson, 1993; Ukonmaanaho and Starr, 2001; Hagen-Thorn et al., 2006). Ukonmaanaho and Starr (2001) is the only study that has measured the in-situ flux of nutrients from leaf litter collecting in litter-traps with the intent to determine if leaf litter nutrient contributions are underestimated. Ukonmaanaho and Starr (2001) positively identified that nutrient cycling studies have underestimated the return of nutrients from leaf litter. Similarly, Oelbermann (1999) conducted an ex-situ study to determine if leaf litter collecting in litter traps, when exposed to precipitation, would undergo weight and nutrient change from leaching. However, Oelbermann (1999) found that, for the most part, leaf litter studies likely overestimate the amount of nutrients entering the system. The literature lacks consensus and a proper investigation into the extent of nutrient modification in leaf litter collected from litter traps.

The purpose of this study is to address the previously mentioned gap in the literature and determine: 'Does the litter trap method produce inaccuracy in leaf litter studies?' Foremost, this study will determine whether sugar maple (*Acer saccharum* Marsh.), American basswood (*Tilia americana* L.) and American beech (*Fagus grandifolia* Ehrh.) leaf litter collected from litter traps after a two week period undergo change in comparison to freshly abscised leaf litter.

The specific objectives of the research were to:

1. Determine the effect of in-situ litter trap conditions on leaf litter dry weight and nutrient content (C, N, P, K, Ca and Mg).

2. Determine the effect of precipitation on leaf litter dry weight and nutrient content (C, N, P, K, Ca and Mg).

3. Determine how different levels of precipitation effect leaf litter dry weight and nutrient content (C, N, P, K, Ca and Mg).

4. Determine the effect of freeze-thaw action on leaf litter dry weight and nutrient content (C, N, P, K, Ca and Mg).

5. Determine the effect of microbial activity on litter collected in litter-traps.

6. Compare differences in litter degradation between three species of deciduous trees.

The anticipated outcome of this study is to devise a correction that would take into account the change in the nutrient content of leaf litter collecting in litter traps so to provide an accurate representation of freshly abscised leaf litter. It is hypothesized that leaf litter studies have historically underestimated the nutrient content of leaf litter due to failure to account for lost nutrients from climatic and microbial degradation while left in litter traps.

The remainder of this thesis consists of four chapters each addressing the specific objectives noted above. Chapter 2 will investigate the decomposition of leaf litter in litter traps under in-situ conditions over a 14-day period. The purpose of chapter 2 is to identify if the nutrient content and dry weight of sugar maple (*Acer saccharum* Marsh.), American basswood (*Tilia americana* L.) and American beech (*Fagus* grandifolia Ehrh.) leaf litter is influences by in-situ litter trap conditions. This chapter's findings will provide the insights necessary to determine the potential sources of nutrient and dry weight change. Based on the knowledge gained through chapter two, the following three chapters further investigate the influence of precipitation (Chapter 3), freeze-thaw (Chapter 4) and microbial activity (Chapter 5) on the change in dry weight and nutrient content of leaf litter in litter traps under ex-situ conditions. Chapters 3, 4 and 5 provide a specific Introduction (literature review), Material and Methods, Results and Discussion, and Summary and Conclusion sections relevant to the objectives of that chapter. The results of chapters 2 through 5 will be synthesized in the Final Summary and Conclusion chapter. The results of each of these chapters will merge to form a complete picture of the changes in dry weight and nutrient content of leaf litter in litter traps.

1.7 Study Site

This study was conducted in the Laurel Creek Conversation Area (LCCA), which is owned and managed by the Grand River Conservation Authority. It is located in the northwest corner of the City of Waterloo, 43° 27' N and 80° 22' W, and is 317 metres above sea level (Figure 1.3). Laurel Creek Conversation Area stretches over 293.3 hectares and is divided into the Nature

Centre (LCNC) and Conservation Area (Tupman, 2004). Laurel Creek Conversation Area is in the centre of the Laurel Creek watershed, an area approximately 74 km^2 in size, located within the boundaries of the Region of Waterloo. Within the watershed are a variety of land-uses from farming to woodlots. However, the majority of LCCA is located within the City of Waterloo and therefore greatly influenced by urban pressures. Specifically, the area surrounding the LLCA is under rapid urban development. Almost all of the area is bordered by residential development. The majority of LLCA was acquired by 1965 in order to mitigate flooding. It wasn't until 1975 that 48.5 hectares which now forms the LCNC lands were purchased by GRCA to act as a drainage buffer. At that time the area was of rural designation, but since has been managed as a growing woodlot used mainly to provide outdoor education to the Public and Catholic school boards.

Today, the LCNC is designated as an upland hardwood vegetation community of intermediate maturity (Oelbermann, 1999). The area has provincially significant wetland along one-side, a subdivision development along another and farmland bordering the remaining portion (Tupman, 2004). Despite efforts to keep the property closed from public use outside normal working hours, a boardwalk constructed to facilitate the Nature Centre's educational mandate, attracts a large number of the local residence. From observational evidence, it is clear that the community has taken liberties with the forest property and are encroaching off the path. It is the objective of the LCNC management to conserve, protect and enhance the property though conservation and restoration efforts (Tupman, 2004).

Laurel Creek Conservation Area is underlain by Salina bedrock and the surficial geology is largely glacio fluvial ice-contact deposit, modern fluvial deposit and Maryhill till (Tupman 2004). The majority of the area is characterized as sandy loam soil but also includes loam, organic and fine sandy loam areas (Tupman 2004). The soil classification is Grey-Brown Luvisol. The mean annual precipitation is 907.9mm and the average daily temperature is 6.7°C (Environment Canada, 2007).

This study site was chosen as means to increase the applicability of the study. The LCNC is found within the Deciduous Forest region, as designated by Ministry of Natural Resources

(Watkins, 2006). LCNC forest provides a good representation of forest found within this region, which is widespread in the eastern United States, as well as the neighbouring forest-region entitled Great Lakes St. Lawrence Forest (Watkins, 2006). These areas are dominated by tolerant hardwoods and mixedwood stands (Watkins, 2006). Both of these regions, being located within southern Ontario, are under great development stress (Watkins, 2006). Forests within these areas are characterized as small woodlots adjacent to farmland, fragmented and have been greatly modified by human activities (Watkins, 2006). In total, only 17% of it is covered by forests, most of which is found in areas with low agriculture potential.

The specific species that were studied are sugar maple (*Acer saccharum* Marsh.), American basswood (*Tilia americana* L.) and American beech (*Fagus grandifolia* Ehrh.). Henceforth, *Acer saccharum* Marsh. is referred to as sugar maple, *Tilia Americana* L. as basswood and *Fagus grandifolia* Ehrh. as beech. These three tree species were chosen because they are commonly found in the Deciduous Forest region (Figure 1.2).
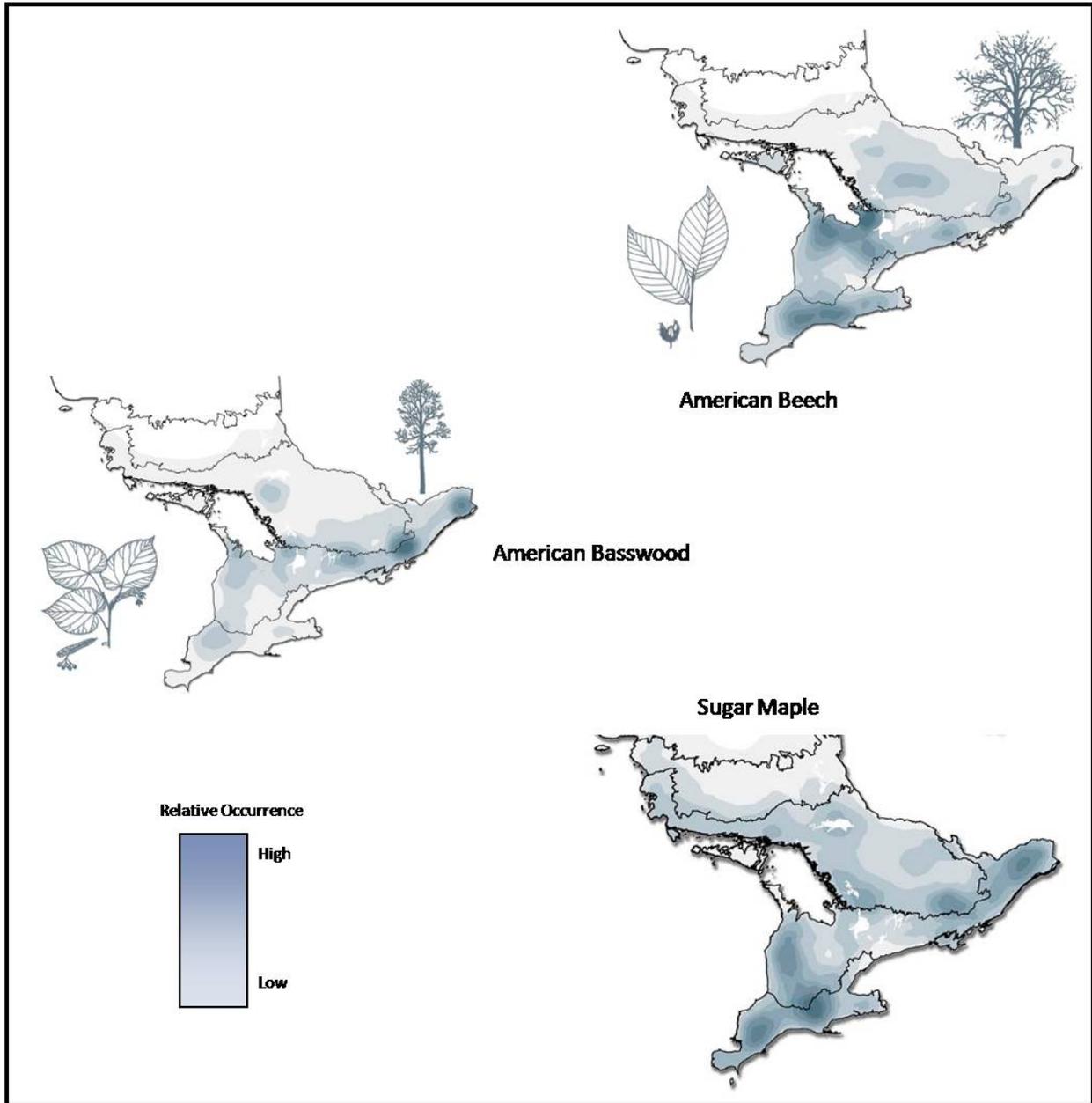
Figure 1.2: Relative abundance of sugar maple (*Acer saccharum* Marsh.), basswood (*Tilia Americana* L.) and beech (*Fagus grandifolia* Ehrh.) trees in Ontario, Canada (Watkins, 2006).
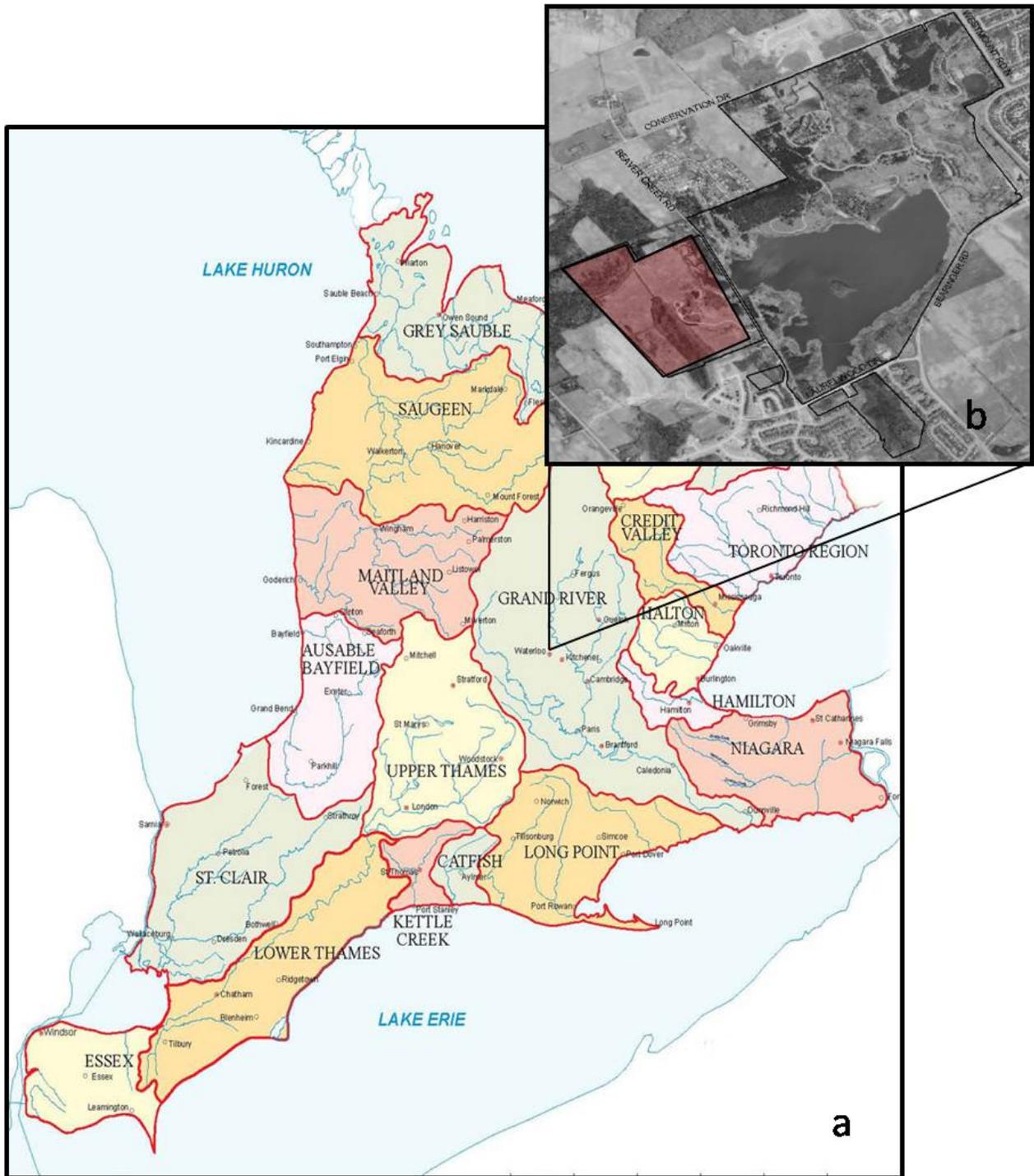
Figure 1.3: (a) Location of Laurel Creek Conservation Area, Waterloo, ON (Conservation Ontario, 2008). (b) Orthophoto of Laurel Creek Conservation Area. Section highlighted in red outlines the Nature Centre (Tupman, 2004).

1.8 General Material and Methods

1.8.1 Leaf litter Collection

Throughout the study the method of leaf litter collection remained constant. Nine study plots within LCCA-Nature Centre were chosen using a Simple Random Sampling technique. The study plot locations were restricted to the upland hardwood community, represented by green shading in Figure 1.4, as the study species were present in this area only. The Nature Centre authority further restricted access to two main areas so as to avoid interference with programmed activities. Those areas are within the boundaries of the circles displayed in Figure 1.4.
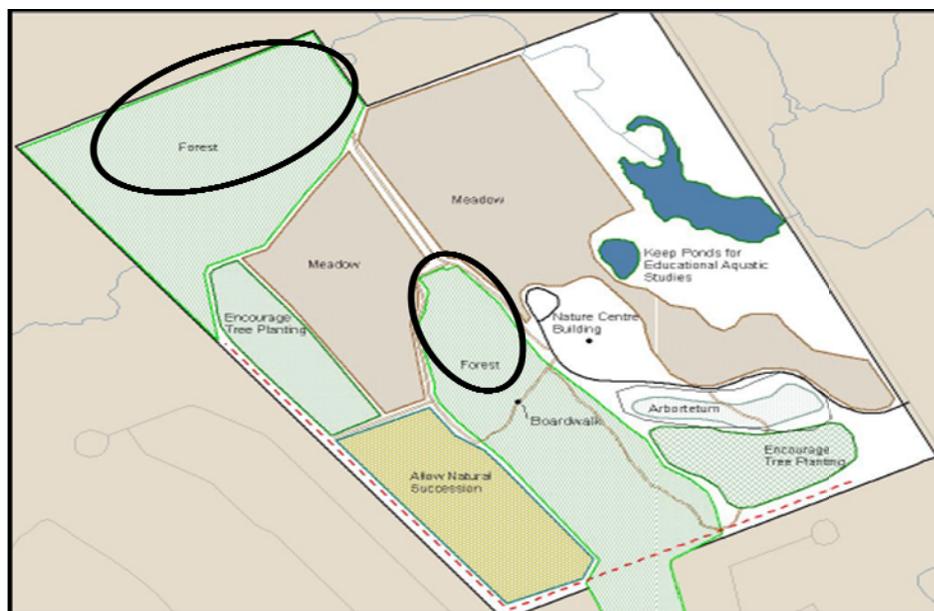
Figure 1.4: Laurel Creek Conservation Area Nature Centre Grounds. The area shaded in green represents the upland hardwood forest. The areas circled are the sections of the upland hardwood forest that the leaf litter was sampled from. Image adopted from Tupman (2004).

The nine study plots were selected using a random number generator. As evident from Figure 1.5, a grid was imposed over a picture of the Nature Centre grounds. Each box represents a 28x30 m area. Nine areas were chosen, three for each of the three species of interest. Within

each area a 10x10 m net was hung from corner posts one-metre above the ground. No part of the net was in contact with the ground. The majority of traps collected mono-species litter, meaning the majority was sugar maple, basswood or beech depending on the site. If litter other than the specie of interest was collected it was discarded in the field while emptying the traps or in the lab before refrigeration.
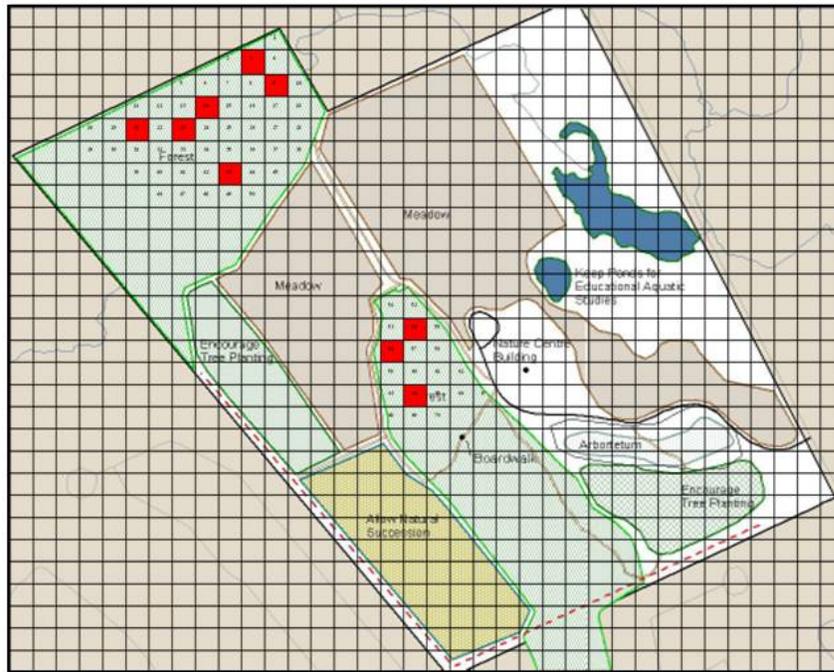
Figure 1.5: The division of the study plot into 28x30 m plots. The areas filled in red represent the leaf litter collection locations chosen randomly. Three areas chosen for sugar maple, three for basswood and three for beech leaf litter collection. Image adopted from Tupman (2004).

Litter fall collection took place throughout the fall, beginning September 20th, 2007 through to October 31st, 2007. Freshly abscised and undamaged leaves were collected daily. This was an integral requirement of the sample collection as it formed a baseline for litter quality levels and ensured the litter did not suffer post senescent leaching. Leaves collecting in the nets overnight or during a rainstorm were discarded. The site was visited twice daily throughout this period, once in the morning to remove litter that had fallen over night and in the evening to

collect litter that had fallen that day. Once collected from the nets, samples were sealed in a polyethylene bag and refrigerated at 4 °C to avert degradation until use (Jones and Steyn, 1973). Upon returning to the lab the samples were sorted and weighed for fresh weight analysis. A sub-sample from each collection was dried at 65 °C for 36 hours for a representative dry weight measurement. The remainder of the leaves were refrigerated for no more than 48 hours before being used for analysis.

1.8.2 Dry Weight Determination

Leaf litter used in experiments was not pre-dried. Drying litter can greatly affect the decomposition in an unpredictable manner. Gessner and Schwoerbel (1989) found that air-dried leaves (alder and willow) lost 20-25% mass, while fresh leaves did not lose a significant percent of mass. Taylor and Barlocher (1996) suggested that air-drying leaf litter significantly affects the amount of mass lost through short-term leaching, although the amount and direction of change was quite variable. More specifically, Barlocher (1992) found that air drying sugar maple (*Acer saccharum* March.) litter induced an increase in leaching. However, all of these studies measured differences based on litter immersed in water. Taylor (1998) measured the affect of air-drying on the decomposition of alder and aspen litter kept in litter bags on the forest floor. It was found that air-dried litter lost less mass than fresh litter over the long term with significant difference, enough to conclude that air-drying added at least 1 year in order for alder to degrade down to 50% of the original mass. In general, drying litter can damage the physical leaf structure by disrupting the cellular structure or cuticle layer. Lousier and Parkinson (1976) stated that oven drying can harm the continuity of the microbial succession, thus over a short term study a loss of microbial activity will affect the decomposition. As well, oven drying can affect the physiochemical constituents of the litter tissue through a release of nutrients (Lousier and Parkinson, 1976).

Not pre-drying leaf litter was particularly important to keep in mind when preparing litter for microbial analysis. It was suggested by Taylor (1998) that drying litter caused irreversible cell damage making microbial enzymatic attack of cellular structure incapable thus greatly minimizing the microbial community present. Clein & Schimel (1994) also explained that

drought conditions cause microbial communities and success of enzymatic attack to be negatively affected.

To avoid having to determine the dry weight of leaf litter used in experiments a relationship between the fresh weight and dry weight for each species was devised. Samples were taken strictly for dry weight analysis throughout the study period. The fresh weight was measured for each sample and then the dry weight after being in the oven for 36 hours at 60°C.The relationship between the dry weight and fresh weight of these samples was used to determine the unknown initial dry weight of leaf litter used in the experiments based on the fresh weight of the leaf litter prior to experimentation.

Chapter 2

Litter Trap Experiment

2.1 Introduction

The return of nutrients to the soil depends primarily on processes of throughfall, stemflow and leaf-litter (Pedersen andBille-Hansen, 1999). Many studies found leaf-litter to be the greatest contributor of nutrients to the soil (Ukonmaanaho and Starr, 2001; Lousier and Parkinson, 1976; Gray and Schlesinger, 1981), although leaf litter is nutrient poor as a result of retranslocation and/or leaching of nutrients prior to senescence (Taylor and Parkinson, 1988a). However, there exists the possibility for the contribution of energy and nutrients from leaf litter to be underestimated. The collection of leaf litter for subsequent analysis usually requires a litter-trap, set-up under the forest canopy, to catch freshly fallen litter. The litter from the trap is then periodically collected on a biweekly or monthly schedule. Between sampling schedules the litter is likely subject to early stages of decomposition, as litter in traps has been found to leach nutrients (Ukonmaanaho and Starr, 2001). Nutrients leached from litter will become part of the soil nutrient or soil water flux pool (Ukonmaanaho and Starr, 2001).

Specifically, litter collecting in traps can be exposed to early processes of decomposition, such as deterioration by bacteria and fungi, and climatic influence, namely leaching, and freezing and thawing (Leuschner, 2005). Although litter-traps eliminate soil microbial influences, studies have found that before abscission, leaves are already colonized with fungi and bacteria, also referred to as phyllosphere communities (Ostman and Weaver, 1982). Furthermore, many studies have found that in the initial stage of decomposition the majority of mass and nutrients lost are attributed to leaching (Bocock, 1963; Anderson, 1973; Gosz et al., 1973; Prescott 2005; Maclean and Wein, 1978; Ibrahima et al., 1995). Since leaching refers to the removal of leaf-litter constituents primarily by rainfall, dew, mist and fog (Tukey, 1970), it is likely that leaf-litter in litter traps would be equally affected. Likewise, leaf litter in traps can be affected by cycles of freeze-thaw, which been found to have an adverse effect on nutrient mineralization rates and nutrient leaching (Ellis et al., 2006).

As previously mentioned, Ukonmaanaho and Starr (2001) is the only study that has measured the in-situ flux of nutrients from leaf litter collecting in litter-traps with the intent to determine if leaf litter nutrient contributions are underestimated. Ukonmaanaho and Starr (2001) measured the leaching of nutrients from litter collected in a Scots pine and mixed forest stand. Ukonmaanaho and Starr (2001) positively identified that nutrient cycling studies have underestimated the return of nutrients from leaf litter. However, ex-situ studies, specifically Oelbermann (1999), found that, for the most part, leaf litter studies tend to overestimate the amount of nutrients entering the system. Oelbermann (1999) compared the loss of nutrients under four precipitation treatment, 21.0 mm, 49.0 mm and 62.0 mm deionized (DI) water, and 49.0 mm of rainwater, on freshly abscised *Populus* spp. leaf litter. The litter exposed to 49.0 mm (DI and rainwater) and greater had a significant weight gain (Oelbermann, 1999).

2.2 Objective

The objective of this study is to determine if, under in-situ conditions, sugar maple (*Acer saccharum* Marsh. hereafter "sugar maple"), american basswood (*Tilia Americana* L. hereafter "Basswood") and american beech (*Fagus grandifolia* Ehrh. Hereafter "beech") leaf litter in litter traps underwent change in comparison to freshly abscised collected leaf litter. In addition, this study tests whether sugar maple, basswood and beech leaf litter respond differently to the same environmental conditions. Two approaches were used to evaluate the change in the nutrient dynamics of freshly abscised leaf litter: (i) mass loss and change in nutrient (C, N, P, K, Ca and Mg) content of leaf litter during a two week period; and (ii) the leaching of dissolved organic carbon and nitrogen and dissolved inorganic nitrogen. Unfortunately, the conditions of this study did not permit sufficient leachate collection over the study period for dissolved organic and inorganic nutrient analysis.

2.3 Material and Methods

2.3.1 Setup and Analysis

The leaf litter collected on October 14th and 15th 2007 was used in the litter trap experiment that took place over a 14-day period beginning October 16th through to October 30th,

2007. In total, nine litter trap experiments were set-up, three for sugar maple, three for basswood and three for beech. A trap was set-up at each of the nine sampling locations (Figure 1.5). The traps were staked to the ground and stood 40 cm high and had a radius of 38 cm (Figure 2.1). For sugar maple and basswood, 20 g of leaf litter collected at each of the locations was suspended over the trap. However, at this point beech trees had only begun to shed their canopy, as a result, the beech traps contained 15 g (fresh weight) as opposed to 20 g of leaf litter. Netting was placed over the trap opening (and leaf litter samples) to eliminate opportunity for sample to be lost or for additional leaf litter to be added to the trap. Henceforth, the three sugar maple traps will be referred to as M1, M2 and M3 where 'M' stands for sugar maple and the number (1,2,3) denotes the location of the trap. The same identification process will be used on the remaining species, where B corresponds to basswood and Be correspond to beech.

From the leaf litter collected on October 14th and 15th 2007 an additional 10 g sample of leaf litter was taken from each of the collection locations (Figure 1.5). These samples represent the baseline data for this study. These samples were dried at 65 °C for 36 hours, weighed, ground with a Wiley Mill using a 2 mm sieve and then a Retsch Ball Mill. The samples were analyzed for phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) at the Soil and Nutrient Laboratory at the University of Guelph. Carbon (C) and nitrogen (N) analysis was conducted using a Costech Instrument ECS 4010 though elemental combustion analysis.

After the 14-day study period, the litter was returned to the lab, dried at 65 °C for 36 hours and weighed. The samples were ground in a Wiley Mill using a 2mm sieve and subsequently ground with a Retsch Ball Mill. The samples were analyzed for P, K, Mg and Ca at the Soil and Nutrient Laboratory at the University of Guelph. Carbon and N analysis was conducted using a Costech Instrument ECS 4010 though elemental combustion analysis.

Figure 2.1: Pictures of sugar maple litter-trap experiment set up in Laurel Creek Conservation Area Nature Centre. Taken by Cassie Corrigan on October 20th 2007.

2.4 Statistical Analysis

To determine if there is a difference between the two samples, baseline (freshly abscised leaf litter) and post-14-day litter trap leaf litter (following the 14-day study period), a paired t-test was completed. The paired t-test was completed to compare each of the measured parameters, where permitting (in some cases the measured parameter was below detectable limits). These parameters were dry weight, C, N, P, K, Ca and Mg. All data was examined for homogeneity of variances between the two sets of compared data (using the Levene Test) and normal distribution was tested using Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test (Dytham, 2003). The variance of the data is homogeneous, normally distributed and continuous. For all statistical analyses the threshold probability level for determining significant differences was $p<0.05$ and $p<0.10$.

2.5 Results and Discussion

2.5.1 Dry Weight Analysis

In the field litter trap experiment, sugar maple leaf litter was found to have the greatest decline in dry weight over the two week period, a mean percent decrease of 12.46%, whereas the dry weight of basswood leaf litter decreased by a mean of 6.80% (Table 2.1). The percent change in dry weight was determined as the percent difference between the baseline dry weight (defined earlier as the dry weight of freshly abscised leaf litter collected on October 14th and 15th 2007) and the dry weight of leaf litter post-14-day litter trap experiment. Compared to the baseline, the dry weight of sugar maple and basswood differed significantly ($p < 0.05$ and $p < 0.10$, respectively) after the 14-day litter trap experiment. Beech leaf litter decreased minimally (0.18%) displaying no significant difference from freshly abscised litter (Table 2.1). The three beech samples showed considerable variation in the reported final dry weight between the three litter traps, such that one of the samples (Be3) reported an increase of 2.09% (Table 2.1).

Table 2.1: Summary of the percent dry weight change of basswood (B), sugar maple (M) and beech (Be) leaf litter before and after 14-days in a litter traps at each of the study site locations (1, 2, 3). Negative percent change identifies a decrease in weight and a positive percent change identifies an increase. Standard deviation is given in parentheses.

Sample ID		Dry Weight Before (g)	Dry Weight After (g)	% Change in Weight
M1		11.00	9.79	-11.00
M2		10.80	9.49	-12.12
M3		10.60	9.09	-14.25
	Mean:	10.80(0.2)	9.46(0.35)	-12.46
B1		12.20	11.35	-6.97
B3		12.78	11.98	-6.26
B2		12.40	11.51	-7.18
	Mean:	12.46(0.29)	11.61(0.33)	-6.80
Be1		11.46	11.27	-1.66
Be2		11.25	11.14	-0.98
Be3		11.75	11.99	2.09
	Mean:	11.49(0.25)	11.47(0.46)	-0.18

A decrease in weight is generally associated with leaching and decomposition of litter (Nykvist, 1959). Most often the rate of decomposition and mineralization are controlled by temperature, moisture and the chemical and physical quality of the litter (Prescott, 2002). Therefore, observed decomposition of the litter is likely the product of climate, micro-climate and litter quality (chemical composition) (Taylor and Parkinson, 1988a; Knutson, 1997). The following results and discussion investigate the impact of climate and litter quality on the observed decomposition of the leaf litter in the litter traps.

2.5.2 Climatic Influences

Over the two week period that the litter traps were set-up in LCNC the litter was exposed to a mean daily high temperature of 15˚C and an mean daily low temperature of 6˚C (University of Waterloo Weather Station, 2008) (Figure 2.2). During the study period there was a total of

23.2 mm of precipitation, the bulk of which can be accounted for on October 23rd 2007(11.4 mm) (University of Waterloo Weather Station, 2008) (Figure 2.2). The remainder of the days received anywhere from 0 – 4.2 mm of rainfall (University of Waterloo Weather Station, 2008) (Figure 2.2). The temperature was less than or equal to 0˚C only once for an eight hour stretch in the evening between October 28th – 29th. From the observed decrease in mass it can be assumed that these climatic conditions assisted in the decomposition of the litter. As mentioned, the initial stage of decomposition is evident through a loss of mass, most often attributed to leaching (Lousier and Parkinson, 1978; Huang and Schoenau, 1997). Huang and Schoenau (1997) found that the greatest decline in weight in the first month of decomposition, regardless of litter type. The initial rapid weight loss was accredited to leaching and decomposition of water soluble material (Huang and Schoenau, 1997). A rapid loss in mass has also been reported in a number of other studies, including Parsons et al. (1990) who referred to this initial rapid loss as a universal observation. As well, cycles of freezing and thawing have been found to accelerate decomposition, nutrient mineralization and nutrient leaching (a topic discussed in greater detail in Chapter 5)(Campbell et al., 2005; Matzner and Borken, 2008).
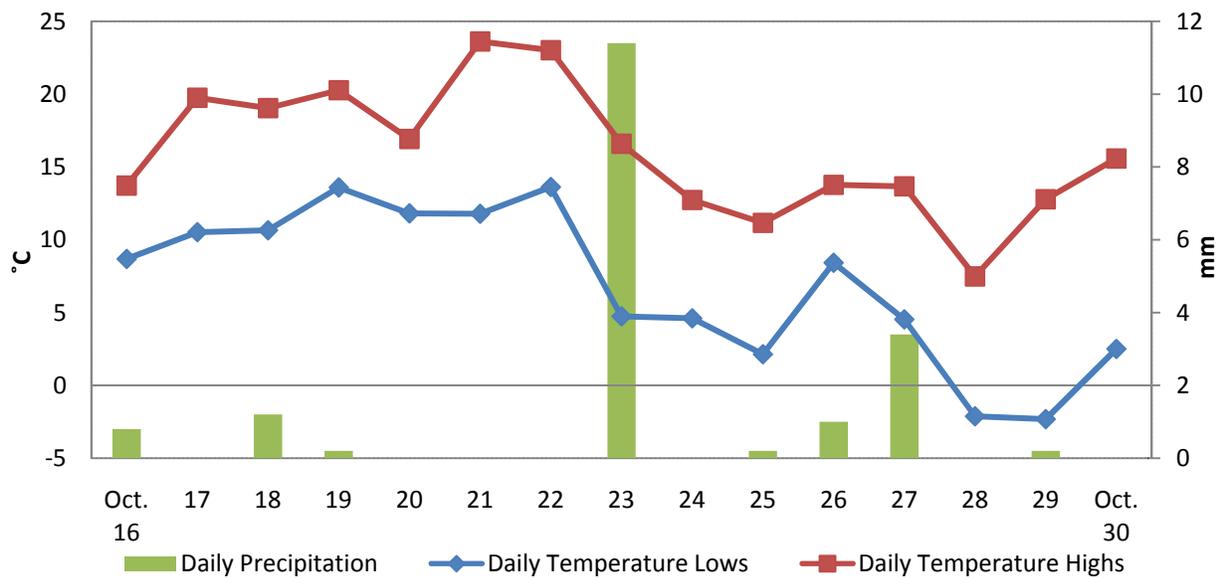
Figure 2.2: Daily high and low temperatures (°C) and daily precipitation (mm) for each day from October 16-30th in 2007 collected at the University of Waterloo Weather Station (43 28' 25.6" N and 80 33' 27.5"W, elevated 334.4m).

2.5.3 Structural Influences

Many studies have also reported that leaf texture affects the decomposition of litter (King and Heath, 1967). Leaf texture is dependent on structural material, mainly lignin, and the thickness and wax content of the cuticle, all of which were found to be inversely related to litter decomposition (King and Heath, 1976). In this study the texture of beech leaf litter was found to be relatively hardier compared to basswood and sugar maple as beech leaf litter did not show structural degradation. Furthermore, leaching characteristics have also been related to leaf toughness and cuticle thickness (Pereira et al., 1998). Leaching is generally slower in tough litter with a thick cuticle (Pereira et al., 1998). This may explain differences in weight lost between sugar maple and basswood, such that basswood may have a thicker cuticle. Taylor and Parkinson (1988b) found that thick and waxy cuticles were better able to resist water absorption, thus allowing for lower initial mass loss rates.

The differences in the three species, although primarily due to differing litter quality characteristics, may also be attributed to differences in canopy cover. First of all, it takes approximately 1-3 mm of rainfall to wet the canopy, ensuring relative saturation, before it is able to reach the ground, or before throughfall and stemflow are initiated (Eaton et al., 1979). Moreover, the amount of rainfall that is intercepted is directly related to the tree species, size and form (Eaton et al., 1979). Besides altering the hydrologic and chemical composition of the precipitation, the canopy can also shade and insulate the ground surface, thus dictating the microclimate (Prescott, 2002). The canopy structure of sugar maple and basswood were quite thin when the study was initiated, and by mid way (first sugar maple and then basswood) the trees were almost fully bare. Basswood trees in this study had no lower canopy, a typical basswood characteristic. Beech did not lose all of its leaves until the very late fall, and some of the lower branches continued to hold foliage until late November. In addition to having foliage later into the season, beech also had a lot of lower branches sheltering the traps. Due to these differences in canopy structure it is fair to assume that the beech litter traps were impacted less by rainfall than the sugar maple or basswood traps. The loss of the canopy can increase decomposition (Edmonds, 1979), primarily due to stimulating optimal microbial conditions, such as increased sunlight and water infiltration (Edmonds, 1991). It is possible that the lack of cover

36

over the study's sugar maple and basswood litter may have stimulated greater microbial growth and thus explaining the greater increase in decomposition, as compared to beech.

As discussed earlier, throughfall is able to alter the chemical composition of the lower reaches of the canopy by depositing nutrients leached from the upper region of the canopy (Hansen, 1996). This has been observed to also extend to litter collecting in litter traps (Ukonmaanaho and Starr, 2001). Although throughfall was not measured, it is noteworthy to mention that the leaf litter may have been affected by throughfall, particularly beech litter trap #3 which exhibited an increase in weight. The areas surrounding beech litter trap 1 and 2 were dominated by beech trees, where as beech trap 3 (Be3), although located under a beech tree, had neighbouring sugar maple trees. The throughfall from the sugar maple trees, likely enriched in comparison to beech, may have deposited nutrients.

2.5.4 Nutrient Analysis

Although all of the litter would have been exposed to the same climatic conditions, differences in the extent of decomposition, measured as a change in mass, still persist. This is likely related to the nutrient content of the leaf litter, often referred to as the litter quality. The quality of the litter is often expressed as a ratio of C to other elements (Flanagan and Van Cleve, 1983). Nitrogen and P are most commonly used as they often limit microbial activity (Taylor et al., 1989). The results of this study found that P did not provide a good comparative measure as nutrient analysis of sugar maple and beech found P to be below detectable limits, however N was measured in all three species.

The results of this study indicate that basswood had the lowest initial C/N ratio, followed by beech and then sugar maple. The mean initial C/N ratio for basswood, beech and sugar maple were 37.15(1.37), 58.60(1.69) and 69.17(0.25), respectively. Although many studies found the C/N ratio negatively correlated to decomposition, this study did not find the initial C/N ratio to be correlated to the observed loss in weight. This may suggest that it is the observed shifts in the C/N ratios during the study period that is responsible for leaf litter weight loss. The C/N ratio post-14-day study period found basswood to be the lowest, followed by sugar maple and then beech. The post-14-day C/N ratio for basswood, sugar maple and beech are 37.36(1.25),

65.31(0.51) and 71.83(1.37), respectively. The change in the C/N ratio observed during the study was an increase of 21.22% for beech and 0.80% for basswood. While sugar maple C/N ratio decreased by 5.57%. Therefore, it appears that the decrease in C/N ratio is positively correlated to the loss of dry weight (Table 2.2).

Table 2.2: The relationship between the change in the C/N ratio and dry weight of sugar maple, basswood and beech leaf litter. A negative percent change reflects a decrease and a positive change reflects an increase. Standard error is given in parenthesis.

	Initial C/N	Post-14-day C/N	% Change in C/N	% Change in Dry Weight
Sugar Maple	69.17(0.25)	65.31(0.51)	-5.57	-12.46
Basswood	37.15(1.37)	37.45(1.25)	0.80	-6.80
Beech	58.60(1.69)	71.03(1.32)	21.22	-0.81

The observed change in the C/N ratio in this study emphasizes the importance of the C/N ratio in dictating leaf litter weight loss. Similarly, Swift et al. (1979) stressed the importance of substrate quality of different species in dictating decomposition rates. Many studies have attributed the decomposition of litter to the C/N ratio (Aerts, 1997; Flanagan and Van Cleve, 1983; McClaugherty et al., 1985; Taylor et al., 1989; Huang and Schoenau, 1997). The decomposition of litter is often limited by insufficient nutrient levels (Gosz et al., 1973). As previously stated, this is specifically important with respect to N as it is found to most significantly limit microbial production (Taylor et al., 1989). In Melillo et al. (1982) the decomposition of litter was found to be highly negatively correlated to the ratio C to N (C/N ratio).

Taylor et al. (1989) stated that C/N ratios >30:1 initiate N to be immobilized, while ratios <30:1 cause N to be mineralized. All of the litter in this study had higher C/N ratios suggesting that N should be immobilized. However, N levels were found to decrease, most noticeably in beech which had an initial (58.60) and final (71.03) C/N ratio well above 30:1 (Table 2.2). The decrease in N in basswood and beech litter types, and the decrease in C in basswood and sugar maple litter may be attributed to initial losses from leaching. The change in C and N

38

concentrations caused sugar maple litter to become more decomposable, basswood to essentially remain the same, and beech to become considerably less decomposable (Table 2.2).

This study found the post-14-day C/N ratio of basswood to be the lowest, followed by sugar maple, and then beech (Table 2.2). Many studies have reported basswood and sugar maple to have similar decomposition rates, both of which have been reported to be greater than beech (Gosz et al., 1973; McClaugherty et al., 1985; Joergensen and Meyer, 1990). However, basswood had a much lower C/N ratio than sugar maple but did not exhibit as great of a decrease in mass. The difference in weight change between sugar maple and basswood could not be explained by litter quality characteristics, therefore it may be that sugar maple litter was significantly damaged and more susceptible to microbial attack, and/or that microbial activity was more prevalent on the surface of sugar maple litter, a topic that will be discussed in greater detail in Chapter 5.

Elemental analysis determined that the Ca content of beech litter significantly ($p<0.10$) decreased after 14 days in the litter trap. Although not differing significantly, all other elements analyzed (C, N, P, K, Ca and Mg) were observed to change, at least minimally, after the 14 day study period in all three species (Figure 2.3). This is with the exception of P in beech which was not detected in either the freshly abscised samples or in the treated samples. With a lack of statistically significant results the use of descriptive analysis was used but with caution.
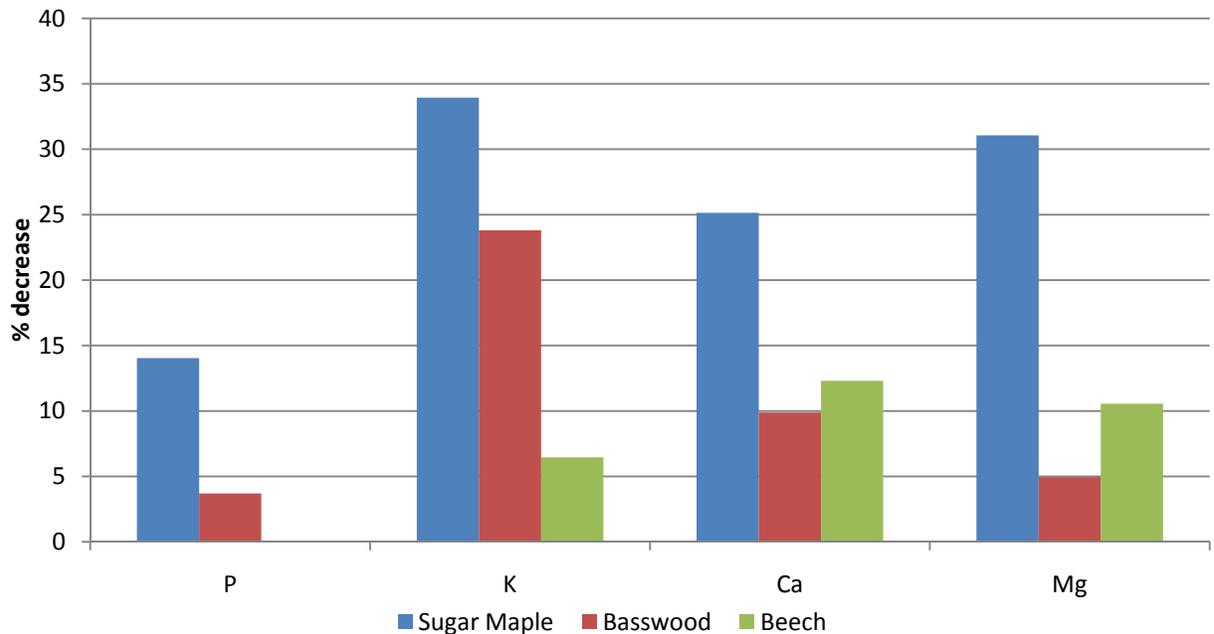
Figure 2.3: The mean percent decrease of K, Mg, P and Ca (measured in mg/kg) in post-litter trap experiment leaf litter as compared to pre- litter traps experiment leaf litter (baseline). Results are the mean of the three litter traps set-up for sugar maple, basswood and beech. Note that K and P in sugar maple and K in beech were below the detection level and so were assumed to be just below the detection limit. This was done to give an idea of the minimalist case.

The K and P content of sugar maple litter and the K content of beech litter collected from the litter traps were below a detectable level (Table 2.4 and 2.5). In order to compare the change in these elements it was assumed that they were just below the detection level (K=0.29 mg/kg and P=0.045 mg/kg). This allowed for minimalist conclusions concerning the effects of leaving leaf litter in litter traps. However, these results are substantiated by the literature as K is readily leached, if not completely, from leaf litter as it is highly soluble (Likens et al., 1994).

Descriptive analysis suggests that the elemental components of sugar maple (i.e. K, Mg, P, and Ca) are affected by litter trap conditions during a two week period. Basswood also showed a decreases in K and Ca, and to a lesser extent, Mg and P (Figure 2.3). Beech exhibiting minimal decreases in K, Mg and Ca as compared to sugar maple and basswood (Figure 2.3). Although, all 3 litter types exhibited change, none of them showed comparable trends. Sugar maple litter

declined, from greatest to least, Mg>Ca>K>P, whereas Basswood litter K>Ca>P> Mg and beech litter Ca>Mg>K (Table 2.4 and 2.5).

The measured dry weight of Be3 was found to increase suggesting that there should not be a demonstrated decrease in other nutrient levels. However, this is not substantiated by the levels of Ca, Mg, P, K and N as they were found to decrease. Although it may be fair to suggest that an increase in weight is the result of additional litter being introduced into the trap from over head canopy, measures were taken to minimize such opportunity. Furthermore, Oelbermann (1999) also witnessed an increase in dry weight of *Populus* spp. Oelbermann (1999) suggested that this may be due to microbial activity.

This study supports the findings of Ukonmaanaho and Starr (2001). Ukonmaanaho and Starr (2001) studied the importance of the loss of leaf litter leachate from litter, accumulating in litter traps, to the total flux of nutrients from leaf litter to the forest floor. Similar to this study, Ukonmaanaho and Starr (2001) found that K significantly leached from the leaf litter while in litter traps. Calcium and Mg also leached but to a much lesser extent (Ukonmaanaho and Starr, 2001). Furthermore, studies on the initial decomposition of litter have found similar changes in litter. For example, Gosz et al. (1973) found that K, Mg and dry weight decreased from the initial point of the study under litter bag conditions. As well, Parsons et al. (1990) found that intact aspen leaf litter lost 14.2% of the original mass after 14 days of being in a litter bag on the forest floor. Huang and Schoenau (1997) found a dry weight decline of 8.9% and 12.4% in Aspen and Hazel leaf litter, respectively, after one month in a litter bag study, where precipitation was 10mm over the entire month. A similar decrease in weight and nutrient levels, in sugar maple and basswood of this study, suggests that litter in litter traps decompose, or leaches, similarly to litter in litter bags. This would then suggest that initial stages of decomposition are not dependent on ground level influences (e.g. soil microbial activity, invertebrate feeding). Therefore, the decomposition of leaf litter in litter-traps is unavoidable.

The results from this study support some of the findings of Oelbermann (1999). Oelbermann (1999) found that, leaf litter exposed to 21.0 mm of deionized water (DI), similar to the 23.2 mm of rainfall in this study, exhibited a decrease in weight, K, N, and P. However,

Oelbermann (1999) observed an increase in weight, N and P when exposed to 49.0 mm of rainfall and 62.0 mm of deionized water (DI). Oelbermann (1999) concluded that leaf litter studies likely overestimate the amount of nutrients entering the system if precipitation is 49.0 mm or greater. Although precipitation levels did not reach 49.0 mm or higher during this study, further investigation into in-situ studies during heavier rainfall is recommended. A noteworthy remark, in Ukonmaanaho and Starr (2001) more than 49.0 mm of rainfall occurred, supporting Oelbermann (1999) results.

Table 2.3: Summary of carbon (C), nitrogen (N) and phosphorus (P) before, pre- litter trap experiment freshly abscised leaf litter, and after, post- 14-day litter trap experiment, C, N and P (measured as mg/kg) for sugar maple (M), basswood (B) and beech for each of the three litter traps. Sugar maple, basswood and beech are abbreviated to M, B and Be, respectively, and the number denotes the location of the trap.

	C Before mg/kg	C After mg/kg	N Before mg/kg	N After mg/kg	P Before mg/kg	P After mg/kg
M1	46.04	45.55	0.67	0.69	<0.050	<0.050
M2	47.31	45.67	0.68	0.71	<0.050	<0.050
M3	45.69	45.94	0.66	0.70	<0.050	<0.050
B1	43.78	45.47	1.27	1.20	0.07	0.06
B2	45.26	45.65	1.16	1.15	0.056	0.062
B3	44.79	45.26	1.18	1.30	0.07	0.06
Be1	48.41	48.62	0.81	0.71	<0.050	<0.050
Be2	48.64	49.48	0.88	0.66	<0.050	<0.050
Be3	48.61	49.52	0.80	0.71	<0.050	<0.050

Table 2.4: Summary of potassium (K), calcium (Ca) and magnesium (Mg) before, pre- litter trap experiment freshly abscised leaf litter, and after, post- 14-day litter trap experiment, C, N and P (measured as mg/kg) for sugar maple (M), basswood (B) and beech for each of the three litter traps. Sugar maple, basswood and beech are abbreviated to M, B and Be, respectively, and the number denotes the location of the trap.

	K Before mg/kg	K After mg/kg	Ca Before mg/kg	Ca After mg/kg	Mg Before mg/kg	Mg After mg/kg
M1	0.39	<0.30	2.04	2.04	0.35	0.33
M2	0.31	<0.30	2.34	2.05	0.39	0.29
M3	<0.30	<0.30	2.28	2.06	0.4	0.29
B1	0.54	0.32	3.67	3.4	0.57	0.47
B2	0.3	<0.30	3.3	3.07	0.53	0.49
B3	0.51	<0.30	4.18	3.64	0.48	0.48
Be1	<0.30	<0.30	1.3	1.2	0.41	0.39
Be2	0.31	<0.30	1.29	1.08	0.39	0.33
Be3	<0.30	<0.30	1.37	1.12	0.37	0.34

2.6 Summary and Conclusions

It was the objective of this study to determine if, under in-situ conditions, deciduous leaf litter in litter traps underwent change in comparison to freshly abscised leaf litter. After 14-days in a litter-trap freshly abscised sugar maple leaf litter exhibited a decrease in weight, C, Ca, Mg and K, while N levels increased. Freshly abscised basswood leaf litter responded similarly, exhibiting a decrease in weight, Mg and K and an increase in C. Basswood leaf litter showed variable N and P results. Freshly abscised beech leaf litter exhibited a decrease in weight, N, K, Ca and Mg and an increase in C. Results from this study confirm that leaf litter collected from litter traps for a 14-day period does undergo change, however the change is variable (Table 2.5).

As well, this study tested whether sugar maple, basswood and beech leaf litter would respond differently to the same environmental conditions. The results of this study suggest that different deciduous leaf liter responds differently to similar environmental conditions. For the most part this is due to different litter quality characteristics. However, leaf litter in litter traps is likely affected by the surrounding environment. Specifically, the results of this study suggest that the overhead canopy may protect and/or add additional nutrients to leaf litter collecting in litter traps.

Although this experiment confirmed that leaf litter in litter traps does undergo change, the mechanism of the change has not been clearly identified. During the study period, the leaf litter was subjected to precipitation and freezing temperatures (Figure 2.2). As well, the loss in weight and nutrient content suggest that microbial activity contributed to the decomposition of the leaf litter. Therefore, the results of this study has inspired further research into the influence of precipitation, freezing temperatures and microbial activity on the decomposition of leaf litter collecting in litter traps. The following three chapters focus on investigating the impact of each of these individually.

Chapter 3
Leaf Litter Leaching Experiment

3.1 Introduction

The decomposition of leaf-litter involves physical and chemical processes that reduce it to its elemental constituents (Aerts, 1997). Although the process involved in decomposition of leaf-litter is shown to vary in description, generally following three main phases: an initial rapid decline in mass due to leaching and loss of soluble components (abiotic breakdown), conditioning by microorganisms, and breakdown through invertebrate feeding and physical abrasion (biotic breakdown) (Berg and Staaf, 1981; Boulton and Boon, 1991). Together these processes mineralize nutrients making elements available for plant uptake (thus controlling plant growth), and humify organic matter maintaining soil organic matter content (SOM) (Lavelle et al., 1993). Besides mineralization, nutrients can also be made available to plants via leaching from leaf litter (Berg and Staaf, 1981). Leaching is considered to be important in only the first few weeks following leaf litter production (Tietema and Wessel, 1994). This was found, by Tietema and Wessel (1994), to be due to a decrease in the amount of leachable solutes in the leaf litter following the initial period of decomposition.

The efficiency of nutrient release (or the rate of decomposition) is dependent on a variety of factors. These factors include micro- and macro-climates (Edwards, 1975), leaf-litter chemical composition and physical state (Swift et al., 1979; Anderson, 1991; Aerts 1999), the quantity (Knutson, 1997) and diversity of leaf litter (Ball et al., 2008). Lavelle et al. (1993) proposed a hierarchal model for the decomposition of litter, suggesting that some factors dictate the decomposition of litter more than others. The levels of the hierarchal model are as follows: climate (temperature and moisture)> physical properties of soil (clay and nutrients)>litter quality> macro and microorganisms (Lavelle et al., 1993). However, many studies have stated a simpler set of dictating factors that influenced the decomposition of litter. These factors are the following: (i) environmental conditions, (ii) the chemical quality of the leaf-litter, and (iii) soil organisms (Swift et al., 1979; Beare et al., 1992; Couteaux et al., 1995; Aerts, 1997).

Continual debates persist on the importance of abiotic and biotic processes in the decomposition of litter. While some confirm that the decomposition of litter is primarily dependent on the substrate quality (Swift et al., 1979l; Flanagan and Van Cleve, 1983), others maintain that temperature and moisture are more important (Bunnel et al., 1977). Taylor and Parkinson (1988c) studied the relative importance of moisture and temperature on decomposition and found temperature to be a considerably more important determinant (Taylor and Parkinson, 1988c). This is in contrast to the many studies that have found temperature and moisture interdependent.

Although decomposition rates will differ depending on the conditions present, almost all litter decomposition follows an exponential decay curve, at least in short term studies (Prescott, 2005). As previously stated, the initial stage of decomposition is typically met by a relatively large decrease in mass which is usually attributed to leaching (Bocock, 1963; Anderson, 1973; Gosz et al., 1973; Prescott 2005; Maclean and Wein, 1978; Ibrahima et al., 1995; Parsons et al., 1990). Leaching refers to the removal of leaf-litter constituents primarily by rainfall, but can also be attributed to dew, mist and fog (Tukey, 1970). Taylor and Parkinson (1988d) determined that the decomposition of aspen (*Populus tremuloides*) litter followed the exponential decay curve. The first two weeks showed a rapid decline of 14% of the total mass resulting from leaching of labile nutrients (Taylor and Parkinson, 1988d). Parsons et al. (1990) found that although aspen litter had rapidly lost mass in the first few weeks of decomposition, the loss was mainly attributed to physical leaching. Two weeks was not sufficient time for microbial populations to establish (Parsons et al., 1990).

Many studies demonstrated the importance of climate, and more specifically, the importance of rainfall to the decomposition of litter, especially in the early stages of decomposition (Swift et al., 1979; Couteaux et al., 1995; Martin et al., 1997). Rainfall was shown to directly impact leaf litter by stimulating the leaching of soluble (labile) compounds and substantially decreasing the mass (Nykvist, 1963; Tukey, 1970; Swift et al., 1979; Martin et al., 1997; Salamanca et al., 2003). This is typically found because leaching is greatest when the concentration of inorganic nutrients (K, Ca, Mg) is high in the intercellular spaces of the leaves, a situation usually present in freshly abscised leaves, as well as rapidly growing leaves (Chapin,

1980). If the rate of nutrients lost is equal to or less than the rate of mass lost then decomposition of organic matter is the cause, but if the rate of nutrients lost is greater than the rate of mass lost it is more likely due to leaching (Gosz et al., 1973). However, the degree of elemental leaching will depend on the nutrient content of the leaves (Chapin, 1980). Many have observed that nutrient leaching is proportional to the age of the leaf, and thus leaching is greatest at senescence (Tukey, 1970).

The intensity and quantity of rainfall influences the rate of leaf-litter leaching in that a lower intensity, more frequent rainfall will increase leaching of leaf-litter (Tukey, 1970). Vanlauwe et al. (1995) found that the decomposition of litter was related to a number of rainfall events, whereas no relationship could be made with the quantity of rainfall (varying from 15-159 mm) on the rate of decomposition. This is further corroborated by Salamanca et al. (2003) study where it was concluded that only a lack of rainfall, even over a short period time and when soil moisture was kept optimal (threshold limit for microbial activity), reduced the rate of decomposition of the leaf-litter. Salamance et al. (2003) found that full or partial application of rainfall showed similar rates of decomposition. Lensing and Wise (2006) found that when precipitation is increased and decreased in relation to current ambient conditions, litter decomposition declines. However, rainfall levels below ambient conditions significantly decreased decomposition (Lensing and Wise, 2006). Yet, some researchers have argued that rainfall doesn't significantly affect litter decomposition. Martin et al. (1997) studied the long term decomposition of *Quercus pyrenaica* in a temperate Mediterranean site from which it was concluded that rainfall did not seem to decisively affect decay, but was more influenced by soil moisture. Martin et al. (1997) suggested that because soil moisture is correlated with rainfall the variables that influence decomposition can be masked.

Quantifying the effect of leaching in the initial stage of decomposition is difficult. It is challenging to separate loss due to leaching from loss due to microbial activity, both of which are influenced by rainfall (Smith and Brown, 1994; Vanlauwe et al., 1995). To minimize the interference of microbial activity in measuring the affect of precipitation, Smith and Brown (1994) suggest treating foliage with rainfall over short periods of time or measuring the leachate chemical content. As well, most leaching studies are conducted by submerging leaf-litter in

47

water (Nykvist, 1963; Ibrahima et al., 1995). This can alter the physical structure of the leaf-litter (Salamanca et al., 2003) and is not representative of the effects of rainfall on leaf-litter decomposition.

3.2 Objective

As previously stated, leaching of nutrients is believed to significantly affect leaf litter in litter traps. The objective of this study was to measure the change in dry mass and nutrient content of deciduous leaf litter, specifically sugar maple (M), basswood (B) and beech (Be), exposed to precipitation. Two approaches were used to evaluate the change in the nutrient dynamics of freshly abscised leaf litter: (i) mass loss and change in nutrient (C, N, P, K, Ca and Mg) content of leaf litter during a two week period; and (ii) the leaching of dissolved organic carbon (DOC) and nitrogen (DON) and dissolved inorganic nitrogen (DIN). The intention was to evaluate the response of leaf litter to precipitation, and determine if sugar maple, basswood and beech leaf litter respond differently to precipitation. Multiple precipitation levels were tested to determine a relationship between leaching and rainfall event magnitude.

3.3 Material and Methods

3.3.1 Leaf-litter Collection

The leaf litter sampling procedure for the leaching experiment was the same as that discussed previously in the General Material and Methods section (Chapter 1). Freshly abscised leaf-litter, collected between October 22nd and 24th 2007, was used in the laboratory leaching experiment. Sub-samples of 20 g (fresh weight) were divided into triplicates (n=3) for each treatment and species. A total of 36 individual experiments were conducted (3 species x 4 treatments x 3 replicates).

3.3.2 Treatments

Four treatment levels were tested – control (Ctrl), low (L), medium (M) and high (H). Total precipitation data for the month of October from 1981-2006 was used to determine the mean L, M and H treatments as 30 mm, 60 mm and 100 mm, respectively (Figure 3.1). The precipitation treatments were determined by dividing the data into three categories – L, M and H

– and then finding the mean within each of the categories (Figure 3.2). The Ctrl treatment received no rainwater exposure (0mm).
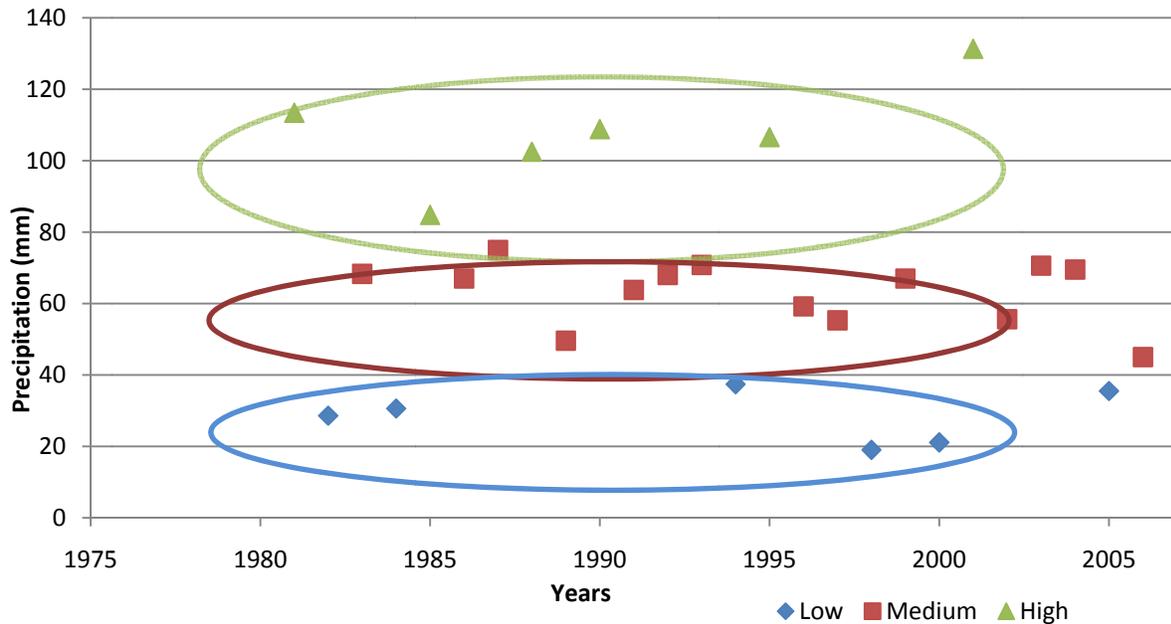
Figure 3.1: Total precipitation data in mm for the month of October from 1981-2006 from the Waterloo-Wellington Meteorological Station (Waterloo Wellington A, Location: 43°27'N, 80°23'W, 317 m elevation, Environment Canada, 2007).

Figure 3.2: An example of the calculation used to determine the daily treatment of precipitation for treatment M (60 mm).

3.3.3 Rainwater Collection

Rainwater was collected in a large circular shaped tub (Diameter =1.50 m, height= 0.50 m) that was placed outside during each rainfall event in an open area with no overhead canopy present to avoid throughfall or stemflow nutrient enrichment. Care was taken to clean the catchment container before and after each collection period with deionized water. The rainwater was stored in 2 L bottles and frozen within one hour of collection. Twelve hours before application, the rainwater was taken out of the freezer and kept under dark conditions to thaw (daily at 9 pm). Once thawed, the rainwater from each 2 L container was mixed together (daily at 9 am). It was found necessary to mix the rainwater to ensure that each treatment was exposed to a heterogeneous application of rainwater. Rainwater was collected throughout September and October of 2007 and was applied as treatment precipitation throughout the two week experimental period. Rainwater was analyzed for pH, DOC, ammonium (NH_4^+), nitrate (NO_3^-) and total Kjeldahl nitrogen (TKN).

3.3.4 Experimental Set-up

The 20 g samples of leaf litter were suspended over a plastic tub (0.32 x 0.27 x 0.12 m) with netting. The netting allowed leachate to percolate through and collect in the tub (Figure 3.3). Each day, the respective treatments (Ctrl, L, M, H) were applied from approximately 9-10 am. From 10-11 am, leachate was collected, filtered and frozen immediately for later analysis. Samples collected for DOC analysis were not frozen and instead were kept in the dark at 4 °C. Rainwater was applied via a watering can at a low flow rate. Care was taken to ensure the rainwater was applied evenly over the litter. One hour provided enough time for the rainwater to percolate through. Leachate was collected on days 1, 6, 10 and 14 over the two-week experiment.

Figure 3.3: Experimental set-up of leaf litter leaching with exposure to 0 (Ctrl), 30 (L), 60 (M) and 100 mm (H) to evaluate changes in litter nutrient content and DOC, DON and DIN or the leachate. Photo taken by Cassie Corrigan.

3.3.5 Sample Analysis

After the 14-days study period, the litter was dried at 65 °C for 36 hours. The samples were ground in a Wiley Mill using a 2 mm sieve and subsequently ground with a Retsch Ball Mill. The samples were analyzed for phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) at the Soil and Nutrient Laboratory at the University of Guelph. Carbon (C) and nitrogen (N) analysis was conducted using a Costech Instrument ECS 4010 through elemental combustion analysis. Leachate was analyzed for NH_4^+, NO_3^-, and TKN using continuous-flow colorimetry (AAIII Bran-Luebbe) and DOC was analyzed using a Rosemont - Dohrmann High Temperature Total Carbon Analyzer.

3.4 Statistical Analysis

A one-way ANOVA was completed to compare the change in dry weight, C, N, P, K, Ca and Mg between treatments, as well as in comparison to the baseline (freshly abscised leaf litter collected on October 22nd and 24th 2007). All data sets were examined for homogeneity of variances between the two sets of compared data (using the Levene Test) and normal distribution was tested using Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test (Dytham, 2003). The variance of the data is homogeneous, normally distributed and the data is continuous. For all statistical analyses the threshold probability level for determining significant differences was $p<0.05$ and $p<0.10$.

To determine if there is a difference between treatment leachate concentrations over the study period repeated measures of ANOVA was completed. Repeated measures of ANOVA was completed to compare each of the measured parameters, DOC, DON, NO_3^- and NH_4^+, between treatments over the study period. Sampling time (over a two week period) was the repeated (within subjects) factor and DOC, DON, NO_3^- or NH_4^+ was the main (between subjects) factor. All data sets were examined for homogeneity of variances between the two sets of compared data (using the Levene Test) and normal distribution was tested using Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test (Dytham, 2003). The variance of the data is homogeneous, normally distributed and the data is continuous. Significance levels were adjusted using the Greenhouse-Geisser and Huyuh-Feldt to account for violations in sphericity.

3.5 Results and Discussion

3.5.1 Dry Weight Analysis

The percent change in dry weight varied between treatment and litter type (Figure 3.4). For beech, an increase in precipitation was met by an increase in the change in weight, such that samples receiving treatment H displayed the greatest decline in weight. Treatments Ctrl, L, M and H had a mean percent change of 9.30%, 10.34%, 10.65% and 12.48% (Figure 3.4). The loss of dry weight of sugar maple followed H>L>M>Ctrl, indicating that treatment L stimulated a greater level of leaching then treatment M. For sugar maple, the mean weight of samples from treatments H, L, M, and Ctrl declined by 16.04%, 13.68%, 5.68% and 2.89%, respectively (Figure 3.4). For sugar maple, only samples treated with H significantly (p<0.10) declined in weight in comparison to the Ctrl. Basswood, exhibited considerably different results. The dry weight increased in all treatments. Basswood leaf litter weight increased most in the Ctrl sample and consecutively less in M> L>H (Figure 3.4). Because the increase in weight was observed in Ctrl it can be assumed that the increase is not related to precipitation, and is likely due to other ambient variables that Ctrl controlled for, such as humidity and temperature. Precipitation may have minimized the influence of other environmental factors as the weight increase is inversely proportionate to the amount of rainfall applied. By subtracting the changes seen in Ctrl by the

52

changes seen in the treatment samples, one would assume the difference would be the absolute affect of the treatment on the sample. In this case, the difference would simply be the affect of rainfall on leaf litter (Figure 3.5). The corrected percent change in weight of basswood indicates that H declined the most (13.35%), followed by L (6.87%) and then M (4.54%), identical to the pattern followed by sugar maple (Figure 3.5). Furthermore, by applying this rational to sugar maple and beech, it is found that precipitation did not greatly influence beech leaf litter weight change, while sugar maple leaf litter was still considerably affected (Figure 3.5). In considering strictly the effect of rainwater on leaf litter decomposition (as measured as the relative change in weight, Figure 3.5), basswood and sugar maple responded similarly. Relative to sugar maple and basswood leaf litter, beech leaf litter was affected minimally.

Although this study observed a change in the dry weight in comparison to the initial dry weight of the leaf litter, it is with caution that these results are reported. As mentioned previously, the initial dry weight was determined based on a relationship found between the fresh (wet) weight and the dry weight (Table 3.1). The relationship was found to exhibit relatively low variability (standard deviation) among the measurements. This gives greater credibility to the relationship, however, there is still a possibility that the approximate initial dry weight may be incorrect.

Table 3.1: The relationship between the fresh weight of sugar maple, basswood and beech leaf litter to the dry weight of the leaf litter. The relationship was used to approximate the initial dry weight of the leaf litter. Standard deviation given in parenthesis.

Sugar Maple		Basswood		Beech	
10.00	5.91	10.00	6.60	10.00	7.60
10.00	5.57	10.00	6.10	10.00	7.50
10.00	5.50	10.00	6.20	10.00	7.80
10.00	5.70	10.00	6.39	10.00	7.70
10.00	5.65	10.00	6.30	10.00	7.65
10.00	5.60	10.00	6.35	10.00	7.60
10.00	5.65	10.00	6.40	10.00	7.55
10.00	5.65	10.00	6.35	10.00	7.65
10.00	5.50	10.00	6.30	10.00	7.80
10.00	5.85	10.00	6.30	10.00	7.75
	5.66(0.13)		6.33(0.13)		7.66(0.10)
Fresh-Dry Weight Relationship:	**1.77**		**1.58**		**1.30**
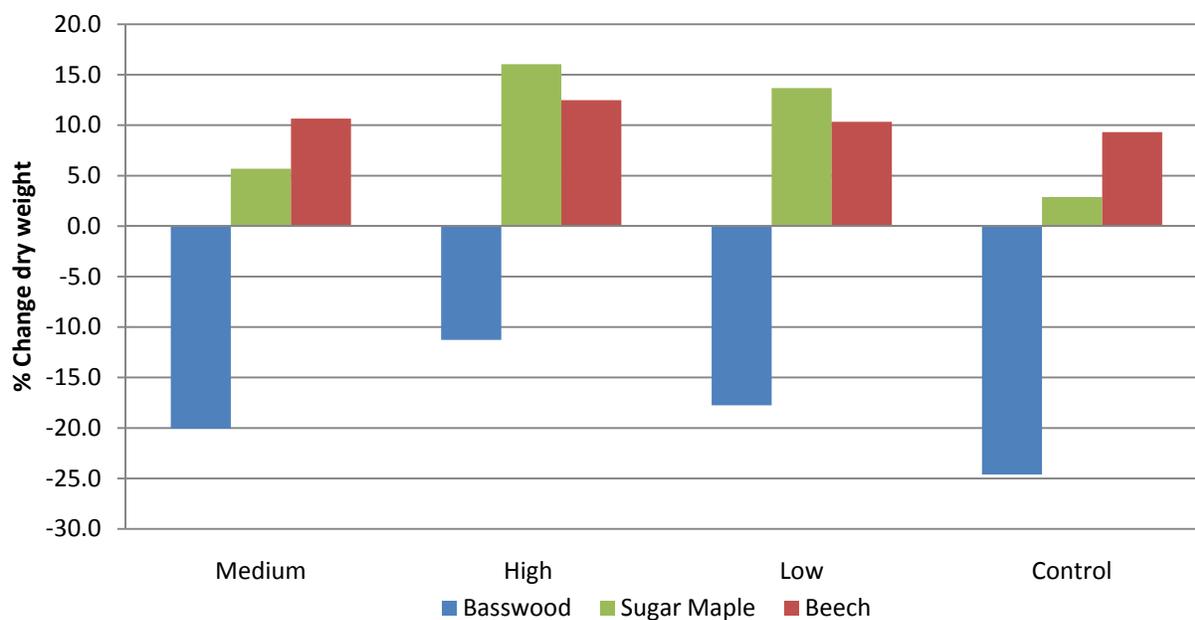
Figure 3.4: Absolute percent change of dry weight (comparison of the initial to final dry weight) for basswood, sugar maple and beech leaf litter after exposure to Medium (60mm), High (100mm), Low (30mm) and Control (0mm) precipitation treatments over a 14-day period. Values above zero identify a decrease in dry weight and values below zero identify an increase in the dry weight.
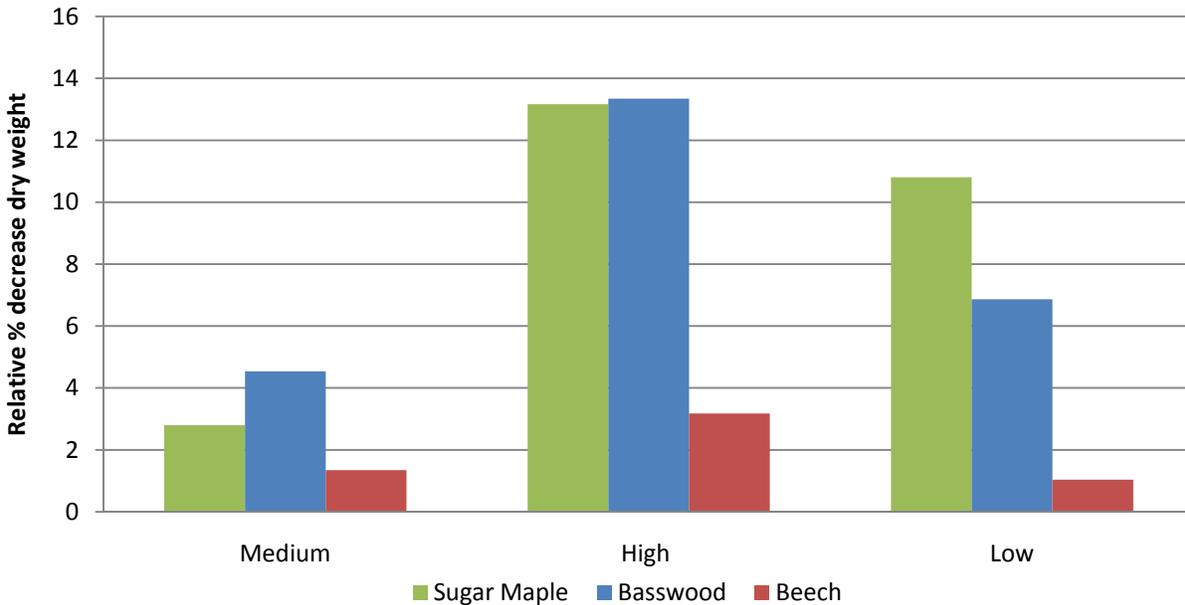
Figure 3.5: The relative percent decrease in dry weight (comparison between control and treatment) for basswood, sugar maple and beech leaf litter after exposure to Medium (60mm), High (100mm) and Low (30mm) precipitation treatments over a 14-day period.

The loss of dry weight of beech leaf litter in response to precipitation is similar to previously reported findings. This study found the loss of dry weight in beech leaf litter directly related to the quantity of precipitation applied. Many studies found supporting evidence that the decomposition of litter is attributed to the quantity of rainfall, at least over the short-term. Salamanca et al. (2003) measured the in-situ decomposition of litter (deciduous broad-leaf forest dominated by *Quercus* species) under varying degrees of exposure to rainfall, namely fully covered, partially covered, and uncovered. It was found that mass loss decreased with reduced exposure to rainfall (Salamanca et al., 2003). Knutson (1997) found increased annual total decomposition of an Eastern Deciduous Forest, dominated by sugar maple, basswood and oak, associated with higher levels of precipitation.

Sugar maple and basswood leaf litter did not exhibit a direct response to precipitation. Precipitation levels higher than,and lower than, medium precipitation induced greater leaching. Lensing and Wise (2007) conducted an in-situ study on litter from an oak-maple-hickory forest to determine if changes in precipitation regimes would affect rates of litter decomposition. The litter was exposed to ambient, below ambient and above ambient precipitation levels (Lensing

and and Wise, 2007). However, results found that above and below ambient precipitation levels retarded the rate of decomposition (Lensing and Wise, 2007). Litter exposed to above ambient conditions decayed 20% slower than ambient and litter exposed to below ambient conditions decayed 78% slower (Lensing and Wise, 2007). This study found that increased precipitation induces increased decomposition among sugar maple and basswood samples. This results was the opposite of the Lensing and Wise (2007) study. Sugar maple and basswood samples exposed to treatment L decayed 8% and 2.33%, respectively, more then compared to samples treated with M. Many studies suggest that mass loss associated with rainfall is due to leaching and microbial activity (Salmanca et al., 2003). This may help to explain why sugar maple and basswood did not behave similar to other studies. Treatment M must have unfavourably influenced the microbial community, eliminating opportunity for microbes to assist in further decomposition.

The increase in weight, experienced by Basswood, has been reported in other studies. Oelbermann (1999) tested four precipitation treatment, 21.0 mm, 49.0 mm and 62.0 mm deionized (DI) water, and 49.0 mm of rainwater, on freshly abscised *Populus* spp. leaf litter. The litter exposed to 49.0 mm (DI and rainwater) and greater had a significant weight gain (Oelbermann, 1999). Although no conclusive explanation could be made, it was suggested that the weight increase may be related to microbial activity (Oelbermann, 1999). Likewise, no clear explanation can be made from this study, except that the increase was likely not related to precipitation as it was also measured in the control. This may suggest that nutrient deposition via rainwater assisted in the weight increase.

3.5.2 Nutrient Analysis

As previously mentioned, two main variables that dictate the decomposition of litter are climate and litter chemistry (Swift et al., 1979; Lavelle et al., 1993). Having already accounted for the affect of climate (i.e. between treatment variations), the influence of leaving leaf litter in litter traps for two weeks may be due to the chemical composition of the litter. Joergensen and Meyer (1990) studied the decomposition of beech (*Fagus sylvatica* L.) leaf litter in an in-situ experiment in Goettingen Forest of Lower Saxony, Germany. Joergensen and Meyer (1990) found the decomposition rates of freshly abscised beech leaf litter to be similar from year to year

although the climatic conditions varied. This suggests that for beech the chemical composition of the litter is a larger determinant of the decomposition rate as opposed to precipitation. In this study beech leaf litter exhibited a decrease in N. This resulted in an increase in the C/N ratio (Figure 3.6 and 3.7). The decrease in N, and thus an increase in the C/N ratio, was most pronounced in M, followed by L>H>Ctrl. These results resemble the dry weight change, in that H lost the most weight and also had the highest C/N ratio. The C content of beech leaf litter did not change over the two week study period.

Similar to the findings of this study, other studies have also reported a low rate of beech leaf weight loss (Joergensen and Meyer, 1990; Mellilo et al., 1982). This has mainly been attributed to a high lignin and low N content (Joergensen and Meyer, 1990). Likewise, many studies have found the decomposition of beech to be slower than that of sugar maple (Gosz et al., 1973; Mellilo et al., 1982). Gosz et al. (1973) determined that after the first month sugar maple had significant mass loss compared to beech, mainly due to leaching. Gosz et al. (1973) concluded that beech was highly resistant to leaching or has fewer constituents to leach. The C/N ratio of beech leaf litter in comparison to basswood leaf litter explains the differences in dry weight results; however, the differences in C/N ratio between beech and sugar maple litter cannot entirely be explained by C/N ratios as they are very similar (Figure 3.6). The difference may be associated with leaching.

Similar to this study, other researchers demonstrated the similarities between sugar maple and basswood decomposition. Page and Mitchell (2008) noted that beech leaf litter had a significantly higher lignin:N ratio and the lower mass loss rate, as compared to both basswood and sugar maple. Basswood leaf litter was reported to have the lowest C:N ratio explaining its relatively high decomposition rate, followed by sugar maple (Page and Mitchell, 2008). Furthermore, Madritch and Cardinale (2007) found that basswood had the lowest C:N ratio and lignin content and the fastest rate of decomposition, although sugar maple decomposed at a very similar rate. This study found sugar maple to lose more weight than basswood leaf litter, however, basswood leaf litter had a much lower C/N ratio (Figure 3.6) suggesting that it should have had a greater decrease in mass.
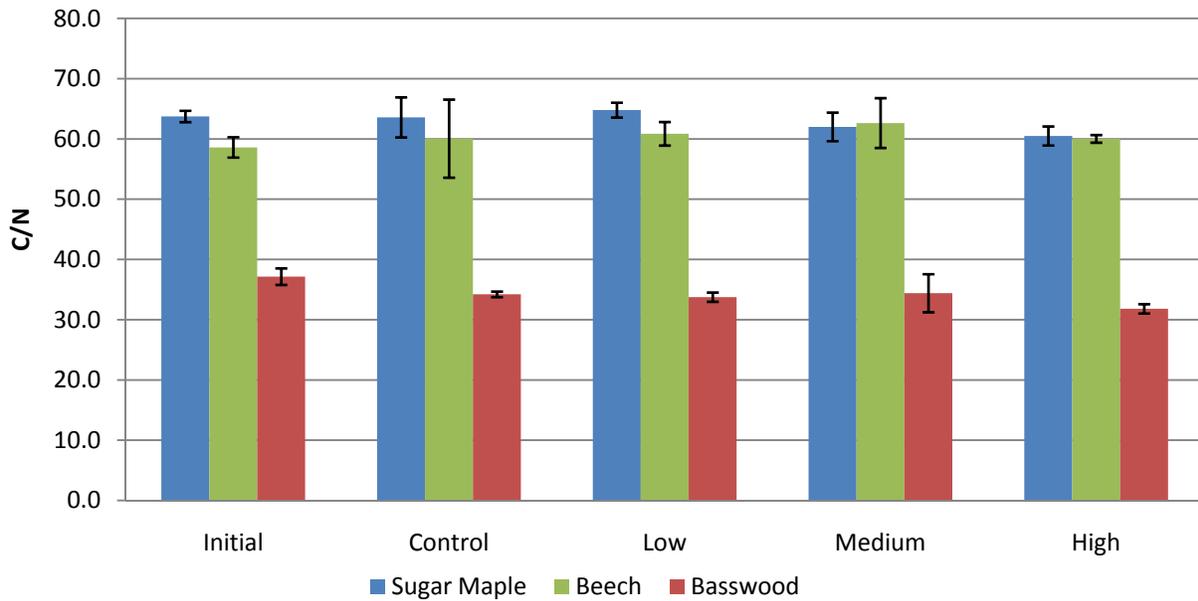
Figure 3.6: Carbon to Nitrogen (C/N) ratios for sugar maple, beech and basswood leaf litter before the pre- leaching experiment (baseline) and post- leaching experiment (after) for each of the treatments (Ctrl, L, M and H).
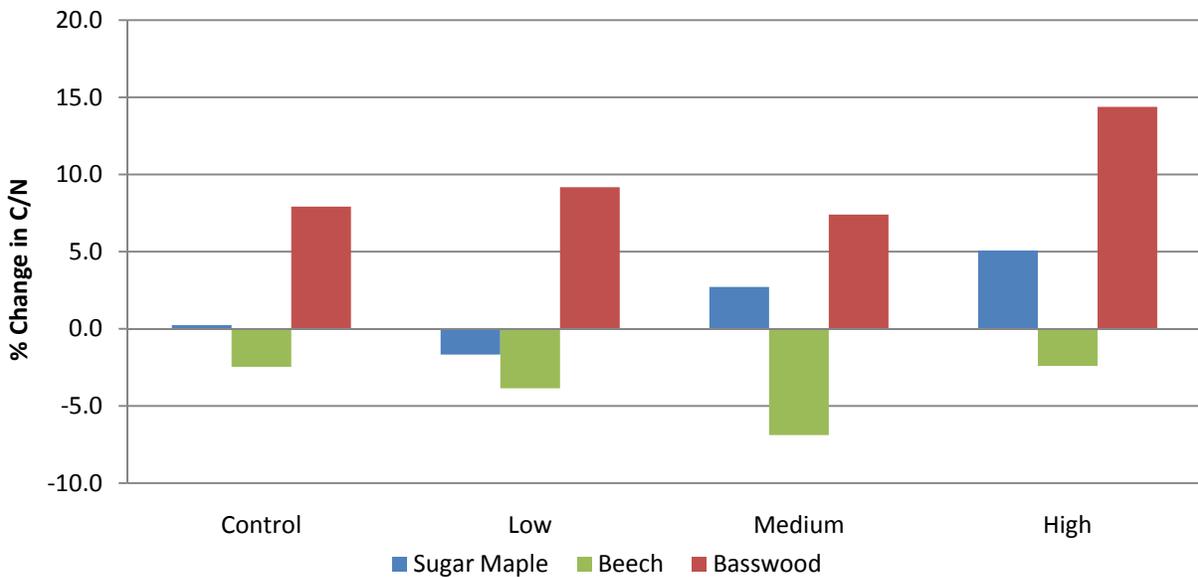
Figure 3.7: The percent change of carbon to nitrogen (C/N) ratios (a comparison of the baseline C/N to the final C/N after the experiment) for treated (Ctrl, L, M and H) sugar maple, basswood and beech leaf litter. Values above zero reflect a decrease in the C/N ratio and values below zero reflect an increase in the C/N ratio.

58

Sugar maple and basswood leaf litter exhibited an increase in the N content of the leaf litter with increased precipitation . This resulted in a decrease in C/N ratio (Figure 3.6). The increase in N is likely from immobilization, as determined by Rutigliano et al. (1998), and/or sequestration of N from rainwater, as determined by Ukonmaanaho and Starr (2001). Rutigliano et al. (1998) studied the factors regulating decomposition of beech (*Fagus sylvatica* L.) and fir (*Abies alba* Mill.) litter in Mount Taburno state (50 km east of Naples). Results determined that the N content increased in the litter due to microbial immobilization and degradation of easily decomposable substances (e.g. carbohydrates). Ukonmaanaho and Starr (2001) found leaf litter in litter traps to be a N sink. This was considered to be due to uptake of N in throughfall by microbes living on the leaf litter (Ukonmaanaho and Starr, 2001). These results do not directly follow the change in dry weight. Samples treated with L lost more weight than samples treated with M, but M showed a smaller ratio. Other studies reported that the lower the C/N ratio the greater the decomposition (Melillo et al., 1982; Flanagan and Van Cleve, 1983; Aber at al., 1990), and these differences were attributed to leaching. Both sugar maple and basswood did not exhibit a change in the C content of the leaf litter.

The unexpected behavior of sugar maple leaf litter, specifically related to the loss of dry weight and the high C/N ratio, is likely attributed to leaching. As stated previously, the initial rapid loss in weight of decomposing litter is often attributed to leaching of soluble compounds (Bocock, 1963; Anderson, 1973; Gosz et al., 1973; Prescott 2005; Maclean and Wein, 1978; Ibrahima et al., 1995; Pereira et al., 1998). The difference in weight lost from leaching between litter types is often associated to leaf toughness and cuticle thickness (Pryon, 1976; Gallardo and Merino, 1993). Leaf toughness and cuticle thickness was not measured in this study. However, the observed behavior suggest that sugar maple may have lost more mass because the leaf structure was not as tough and the cuticle was not as thick as basswood.
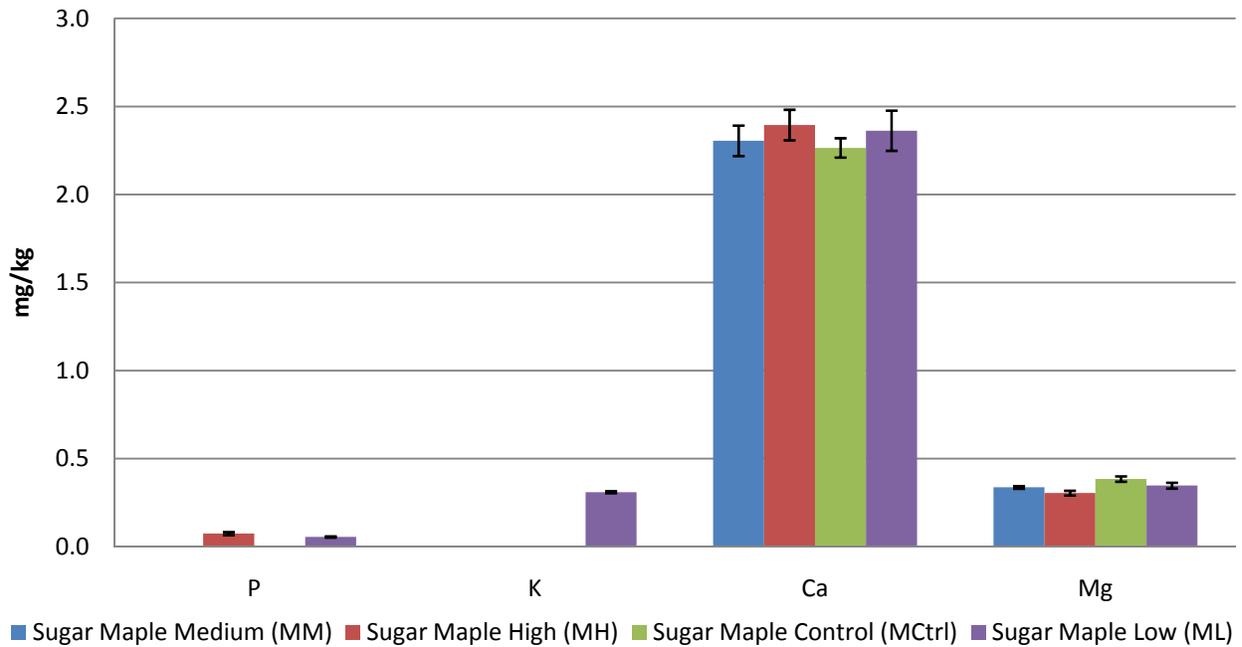
Figure 3.8: The mean sugar maple nutrient content (in mg/kg) of leaf litter after the 14-day study period for medium (M), high (H), low (L) and control (Ctrl) treatments.
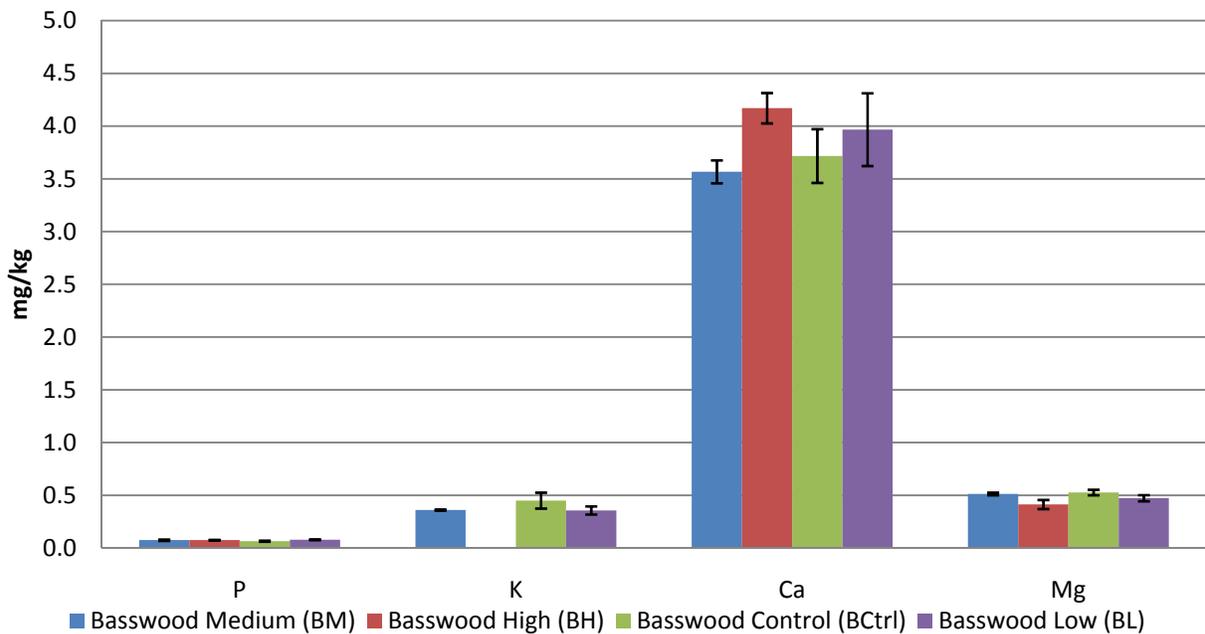
Figure 3.9: Basswood nutrient content (in mg/kg) of leaf litter after the 14-day study period for medium (M), high (H), low (L) and control (C) treatments.

60
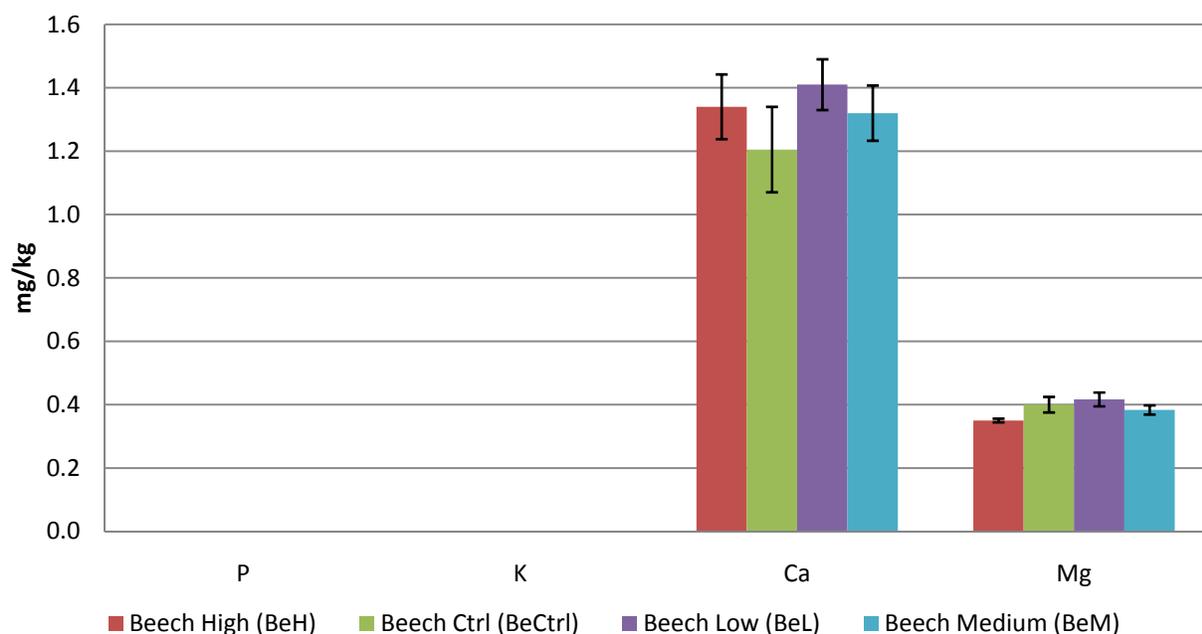
Figure 3.10: Beech nutrient content (in mg/kg) of leaf litter after the 14-day study period for medium (M), high (H), low (L) and control (C) treatments.

For sugar maple (Figure 3.8) and beech (Figure 3.10) K was below detectable limits (0.30 mg/kg) in all treatments and controls. Freshly abscised sugar maple, basswood and beech leaf litter collected for baseline analysis had measurable levels of K of 0.43 mg/kg, 0.45 mg/kg and 0.31 mg/kg, respectively (Table 3.1). This suggests that K is readily lost, even without being exposed to precipitation (as the Ctrl also lost K). Many studies reported that K is readily immobilized from plant material during the initial stages of decomposition (Attiwill, 1967; Tukey, 1970; Waring and Schlesinger, 1985). Gosz et al. (1973) reported similar baseline results. Gosz et al. (1973) reported that K concentrations in sugar maple leaf litter were 0.39 mg/kg, with a standard deviation of 0.14 mg/kg, after decomposing for one month. For beech, K concentrations were measured at 0.25 mg/kg with a standard deviation of (0.04) after one week of decomposition (Gosz et al., 1973). These concentrations are below the detectable limits of this study. Since the baseline concentration of K in both sugar maple and beech leaf litter in this study are within the range reported by Gosz et al. (1973), it is assumed that if measurable, similar finding would be reported in the treated litter of this study. Rutigliano et al. (1998) found K to be very mobile and mainly lost by leaching in early stages of decomposition, specifically in beech leaf litter. Ferrari (1999) found K levels in the fall to be 0.42 mg/kg and 0.44 mg/kg for

61

basswood and sugar maple, respectively. These are very similar to the findings in the freshly collected leaf litter measured in this study. For basswood, only samples exposed to treatment M showed K to significantly ($p<0.05$) decrease in comparison to the control sample (Figure 3.9).

Phosphorus was below detectable levels for all sugar maple and beech samples, both treated and freshly abscised samples (< 0.05 mg/kg) (Figures 3.8 and 3.10). This indicates that P was removed from the leaves before abscission, either retranslocated or leached. More than half of the P in deciduous leaves is re-translocated back to the trees before leaf abscission (Deshesne et al., 2001; Chapin, 1980). For the most part, P is not considered to readily leach from decomposing leaves (Cole and Rapp, 1981), however, P has been found to initially leach and then rely on microbial decomposition (Rutigliano et al., 1998). Tukey (1970) and Qualls et al. (1991) reported P to leach from senescing leaves.

For basswood, P levels were found to increase in comparison to the control (Figure 3.9). Basswood samples treated with L precipitation levels had the greatest increase in P, followed by H and then M (L>H>M). The increase in P decreased the C/P ratio. A decrease in the ratio implies that the element is being accumulated through biological or physiochemical processes (Lousier and Parkinson, 1978), in this case likely sequestered through rainfall. A decrease in the C/P ratio also indicates an increase the decomposability of the litter (Lousier and Parkinson, 1978). The disproportionate increase of P in L treatments to M and H treatments may be due to stresses, such as leaching or washing away of microbes, from excessive water application.

Gosz et al. (1973) reported P concentrations of 0.02 mg/kg and 0.03 mg/kg in freshly abscised sugar maple and beech leaf litter. This is assumed to be similar to the findings in this study as the low concentration reported by Gosz et al. (1971) are below detectable measures and it is may be unlikely that the litter would be completely absent of P. This is further corroborated by the fact that P is found to leach from the litter during early stages of decomposition (Rutiglaine et al., 1998). In a number of studies, P has been reported to increase over the course of litter decomposition, usually over a longer period of time (Gosz et al., 1973; Laskowski et al., 1995; Oelbermann, 1999). The rapid increase in P by basswood may be related to a number of factors. In both Gosz et al. (1973) and Laskowski et al. (1995) the increase in P is explained by

microbial immobilization. The increase in P may also be associated with inputs from rain water. Carlisle et al. (1966) reported that P had been removed from incident precipitation by foliage, as measured by a decrease of P in throughfall.

In all three litter types, treatment H induced the greatest amount of Mg lost (Figures 3.8, 3.9 and 4.10). In both sugar maple and beech, Mg declined proportionate to the level of treatment, hence M>L. However, the opposite is true for basswood. Magnesium is lost more in L samples than in M samples (L>M). The leaching of Mg is strongly influenced by rainfall (Buldgen, 1982). The loss of Mg is attributed to leaching from increased rainwater application. Since the loss of Mg is not equal to the loss of C, the decrease is not related to microbial activity (Staff and Berg, 1982, Laskowski et al., 1995). Mg concentrations in this study are somewhat higher then reported by Gosz et al. (1973). Gosz et al. (1973) reported 0.07 mg/kg in sugar maple and 0.11 mg/kg in beech of freshly collected litter. Over one month Mg changed minimally in both beech, decreasing to 0.10% , and sugar maple, increasing to 0.08%. Freshly collected sugar maple litter in this study reported concentrations of 0.44 mg/kg Mg and decreased over a two week period between 0.30 mg/kg to 0.34 mg/kg. Freshly collected beech litter in this study reported concentrations of 0.40 mg/kg, and decreased over the two week period ranging from 0.35 mg/kg to 0.40 mg/kg. The differences reported in the study may be related to the amount of precipitation the litter was exposed to during this initial phase of decomposition. This study also reports higher levels of Mg in freshly collected sugar maple and basswood than Ferrari (1999) reports. The higher concentration of Mg and the lack of significant difference found in this study may be related to microbial requirement. Elements limiting for microbial growth are often immobilized in litter up to a minimum concentration and then released at a rate equal to the rate of weight loss (Staaf and Berg, 1982).

Calcium increased in all samples, with the exception of basswood M in which it decreased by 2.80%. In basswood and sugar maple, Ca increased most in H, followed by L. Sugar maple treatments H, L and M increased by 5.74%, 4.30% and 1.77%, respectively (Figures 3.8 and 3.9). These differences are much more pronounced in basswood, where basswood H increased by 13.62% and basswood L by 8.08%. The increase of Ca in beech samples was much greater than in either basswood or sugar maple. For beech, L increased the most by an amount of

17.01%, where as beech H increased by 11.20% and beech M by 9.54% (Figure 3.10). It is fair to assume that the increase is primarily related to being exposed to precipitation as freshly abscised Ca levels were similar to the control in all three litter types. Similar to Gosz et al. (1973) K and Mg is lost from the litter at a greater rate than Ca. This is mostly related to the fact that Ca is immobile (Likens et al., 1998). The loss of Ca during litter decomposition in Attiwill (1967) increased over the first three months before subsequently decreasing. Attiwill (1967) found that Ca, along with P and Mg, were lost at rates similar to the loss of dry matter. Gosz et al. (1973) found similar results, although not in as high of concentrations. It is believed that the concentration of Ca in basswood M, although lower than the Ca concentration in freshly collected litter, did not decrease but remained constant. The standard deviation of Ca in fresh leaf litter was relatively high suggesting that the Ca content of the M treated samples are within the measured range (Table 3.1).

In this study, Ca decreased in the control samples of sugar maple by 22.25%, in basswood by 12.20% and in beech by 6.95%. The control samples were not exposed to precipitation and essentially air-dried over the two week period. Without similar finding from other reports, this study is left to suggest that air-drying may cause physical deterioration of the structural components of the leaf allowing Ca to be lost. This is adapted from the understanding that drying litter can cause leaf structural changes (Tukey, 1970).

All treatment samples (H, M, L) from the three litter types (sugar maple, basswood, beech) were compared to freshly abscised samples. In all samples the weight change in sugar maple and basswood were significantly ($p<0.05$) different from freshly abscised samples analyzed directly after collection, whereas, only beech L was significantly ($p<0.10$) different. As well, the K content of basswood litter exposed to M treatment significantly ($p<0.05$) differed from freshly collected litter, along with basswood L P content. Sugar maple exposed to H treatment displayed significantly ($p<0.05$) decreased levels of K (assuming K=0.29 mg/kg) and Mg when compared to the freshly abscised litter. Sugar maple samples treated with L significantly ($p<0.05$) different K (assuming K=0.29 mg/kg) from freshly collected litter, and maple control Mg content significantly ($p<0.05$) differed from the Mg content of freshly abscised

64

litter. In beech samples treated with H, Mg significantly ($p<0.05$) differed when compared to freshly collected litter.

3.5.3 Leachate Analysis

The concentration of DOC, DON, NH_4^+ and NO_3^- in the leachate (rainwater percolating through the leaf litter) were corrected for rainwater DOC, DON, NH_4^+ and NO_3^- concentrations. As well, the measured concentrations of DOC, DON, NH_4^+ and NO_3^- in the leachate were corrected for the quantity of treatment applied (by multiplying the treatment quantity). This was done to correct for a dilution effect. Reported negative values identify when rainwater nutrients were taken up by the leaf litter resulting in a lower concentration in the leachate.

Dissolved organic carbon leached from all treatments throughout the entire study period. The first day of the study, after receiving only one treatment application, exhibited the greatest concentration of DOC in the leachate (Figures 3.11, 3.12, 3.13). Over the remainder of the study, DOC concentrations stayed relatively constant, with little fluctuation. Some treatments developed minor variation to this pattern. Treatments basswood L and sugar maple L leached more DOC after five days, as opposed to the initial treatment (Figures 3.11 and 3.12). Beech litter receiving treatment L, showed the lowest levels of DOC after the fifth day of the study (Figure 3.13). The amount of DOC leached from the litter was proportionate to the quantity of precipitation applied and characteristics of the litter. The greater the precipitation amount applied to the litter the higher the concentration of DOC in the leachate and in total (over the two week period). Hence, treatments H leached more DOC than M, which leached more DOC than L. In addition, sugar maple leached the most DOC over the two week period, followed by basswood, and then beech. Moore et al. (2006) stated that the loss of mass is approximately proportional to the loss of C. In this experiment it was found that the loss of DOC was proportional to the loss of mass, however, the results from C analysis did not relate to this finding.

Godde et al. (1996) reported a similar DOC leaching pattern. Godde et al. (1996) found that DOC concentrations were highest during the initial period of the study and then declined. Following the decline was a period of steady-state within 4to7 days of the litter receiving daily treatment applications (Godde et al., 1996). As mentioned the difference in the amount leaching

is attributed to the amount of precipitation. Michalzik et al. (2001) concluded that, similar to this study, increased precipitation directly stimulates increased DOC. Results from Czech and Kappen (1997) study stated that the greater the repellency of the leaf cuticle to water the less DOC leached. This may explain why beech leached so little DOC in comparison to sugar maple and basswood.
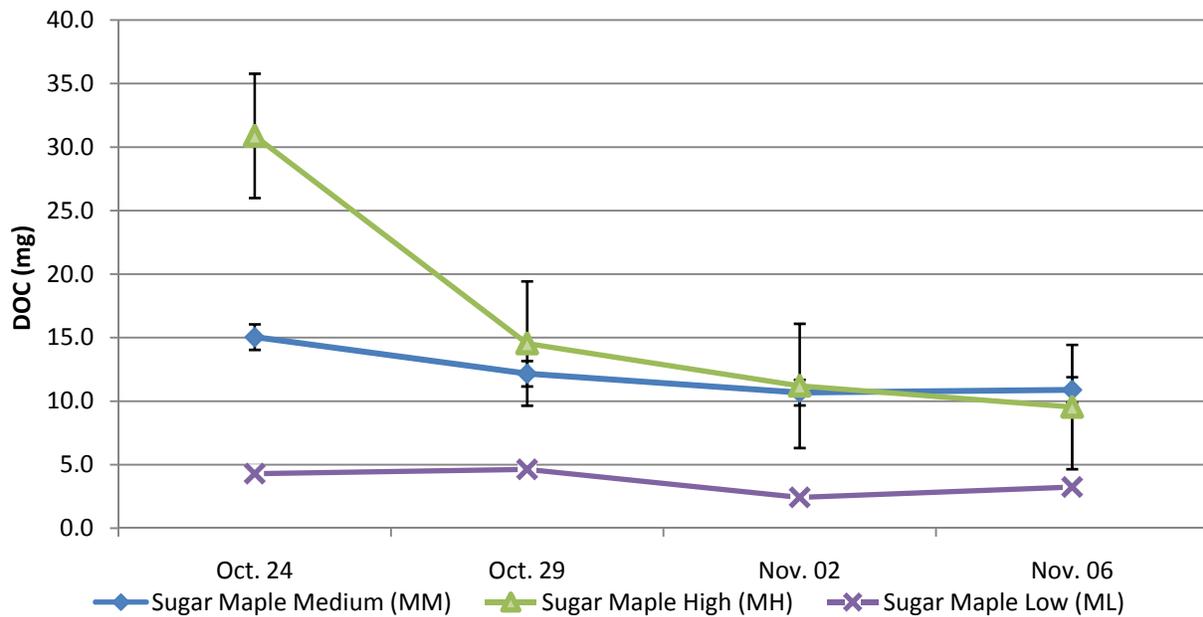
Figure 3.11: Dissolved organic carbon (mg) leached from sugar maple (M) leaf litter when exposed to L (30 mm), M (60 mm) and H (100 mm) precipitation treatments during a 14-day leaching experiment. The DOC concentrations were corrected for rainwater quantity and DOC concentration.
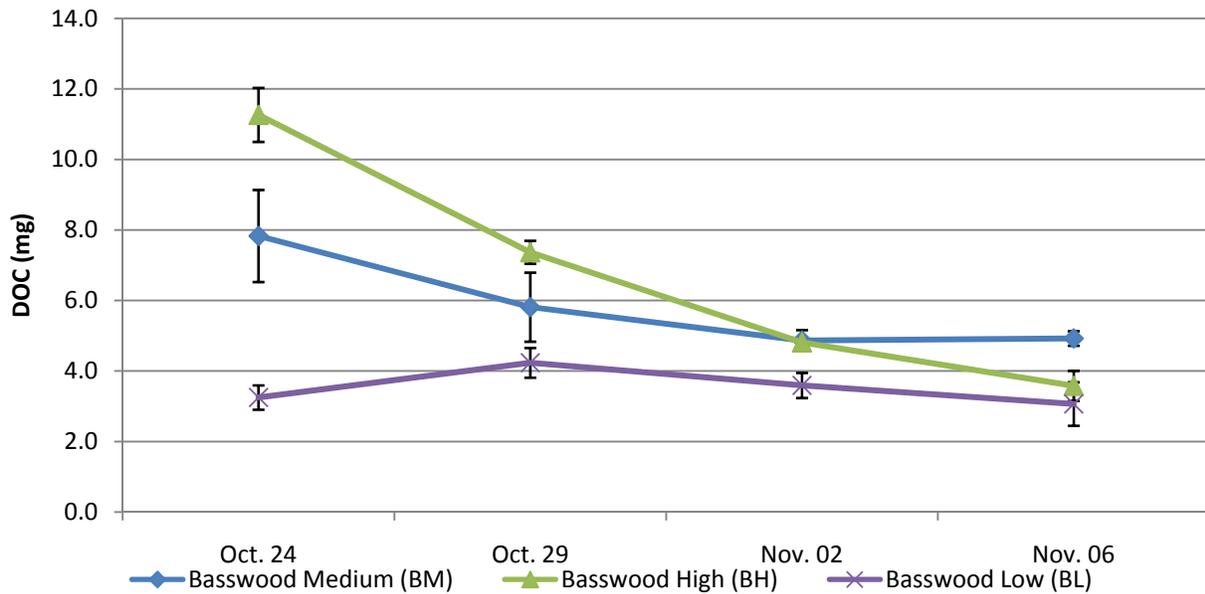
Figure 3.12: Dissolved organic carbon (mg) leached from basswood (B) leaf litter when exposed to L (30 mm), M (60 mm) and H (100 mm) precipitation treatments during a 14-day leaching experiment. The DOC concentrations were corrected for rainwater quantity and DOC concentration.
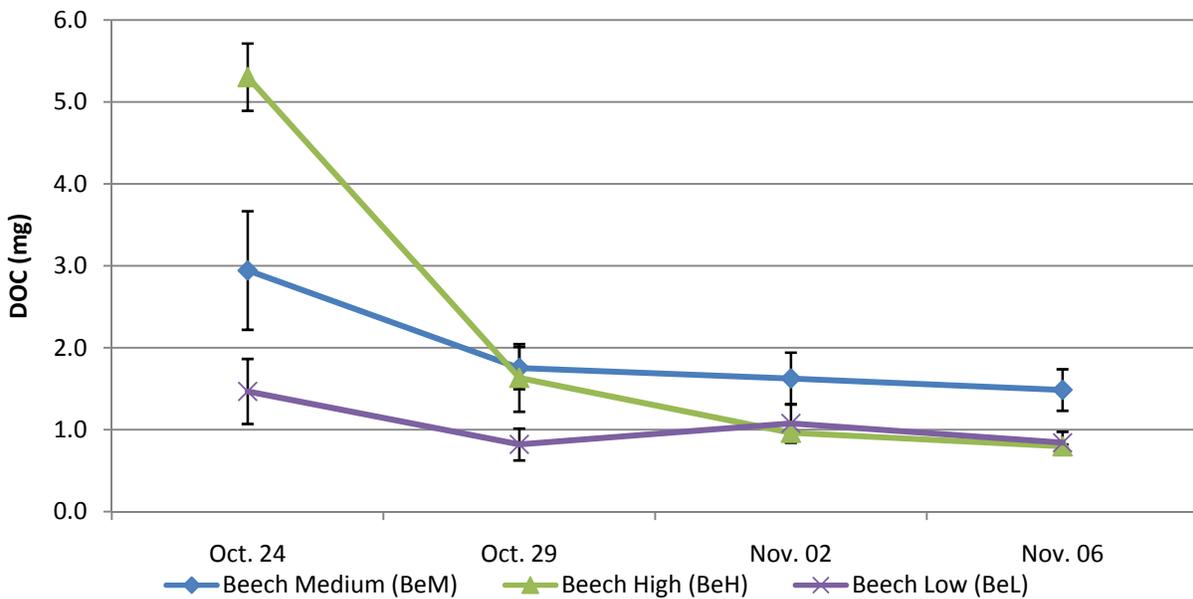
Figure 3.13: Dissolved organic carbon (mg) leached from beech (Be) leaf litter when exposed to L (30 mm), M (60 mm) and H (100 mm) precipitation treatments during a 14-day leaching experiment. The DOC concentrations were corrected for rainwater quantity and DOC concentration.

Qualls et al. (1991) and Michalzik et al. (1999) determined that the majority of DON leached from the forest floor is from the initial soluble substances in leaf litter. Past studies have found leaching of DON responsive to precipitation regimes (Michalizik et al., 2001). Similarly, this study found that DON generally leached more when the litter was exposed to higher treatments of precipitation (Figures 3.14, 3.15 and 3.16). This was most evident in basswood leaf litter (Figure 3.15). Basswood exhibited the greater losses of DON when treated with higher precipitation regimes. Basswood leaf litter leached the greatest concentration of DON initially and then continuously decreased over the two week period. The decrease in the flux of DON can be attributed to an initial loss of more easily removable compounds, followed by a lack of easily removable compounds. Similarly, Cleveland et al. (2004) found that N initially leached when DON concentrations where high, but as the experiment progressed inorganic forms of N dominated. Michalzik et al. (1999) determined that DON and DOC fluxes were correlated and Neff et al. (2003) stated that DOC could be used as a proxy for DON concentrations. In this study basswood DOC and DON followed a comparable leaching trend.

The leaching of DON from beech leaf litter responded more erratically (Figure 3.16). Over the two week period beech leaf litter treated with M leached the most DON followed by H and then L. Beech leaf litter exposed to treatment M exhibited an increase in DON leaching during the two week period. Leaf litter exposed to H and L exhibited inverse leaching results (Figure 3.16). For H, the loss of DON was greatest at the beginning and end of the two week period. For L, the loss of DON was greatest during the middle of the study period. Treatment H showed a similar relationship to inorganic N leaching that Cleveland et al. (2004) reported. For the most part, inorganic N was low when organic N was high. Michalzik et al. (2001) reported that there is no relationship between DON leaching and NH_4^+ and NO_3^- leaching or to the input of NH_4^+ and NO_3^- from rainfall. Leaching of DON from sugar maple leaf litter did not respond to different levels of precipitation as was expected. The only discernable trend that could be obtained from the concentration of DON lost from sugar maple leaf litter was that more DON was lost on day 14 of the experiment than at any other sampling points during the two week period (Figure 3.14). For both beech and sugar maple DON leaching did not relate to DOC leaching.

68
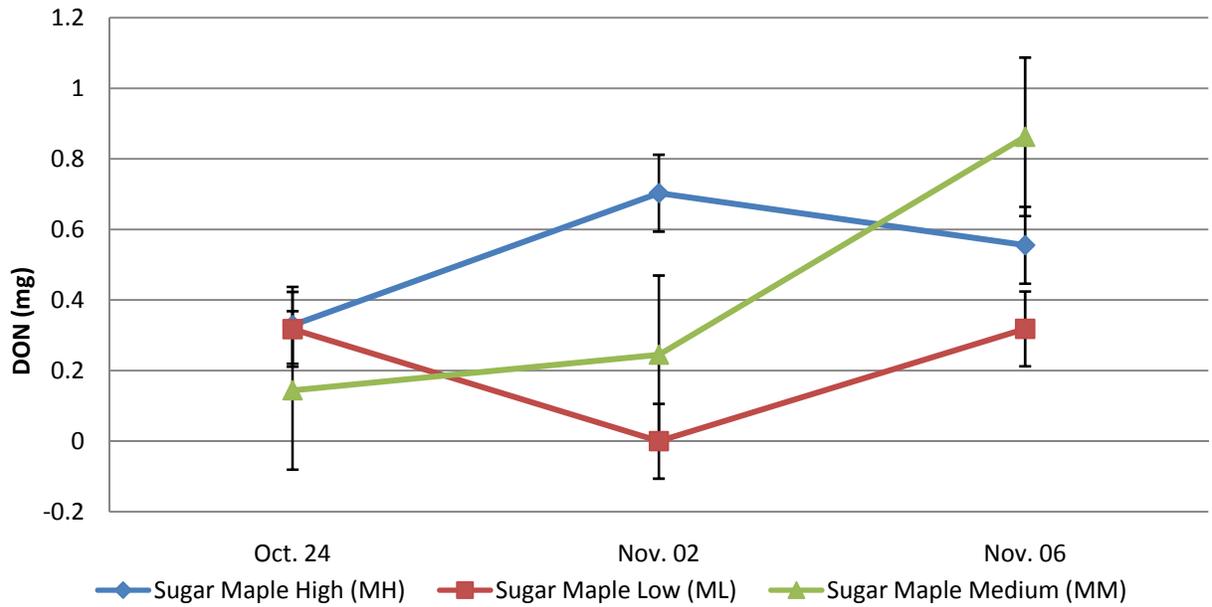
Figure 3.14: Dissolved organic nitrogen (mg) leached from sugar maple (M) leaf litter samples after treated with L (30mm), M (60mm) and H (100mm). The DON concentrations were corrected for treatment quantity and rainwater DON concentration.
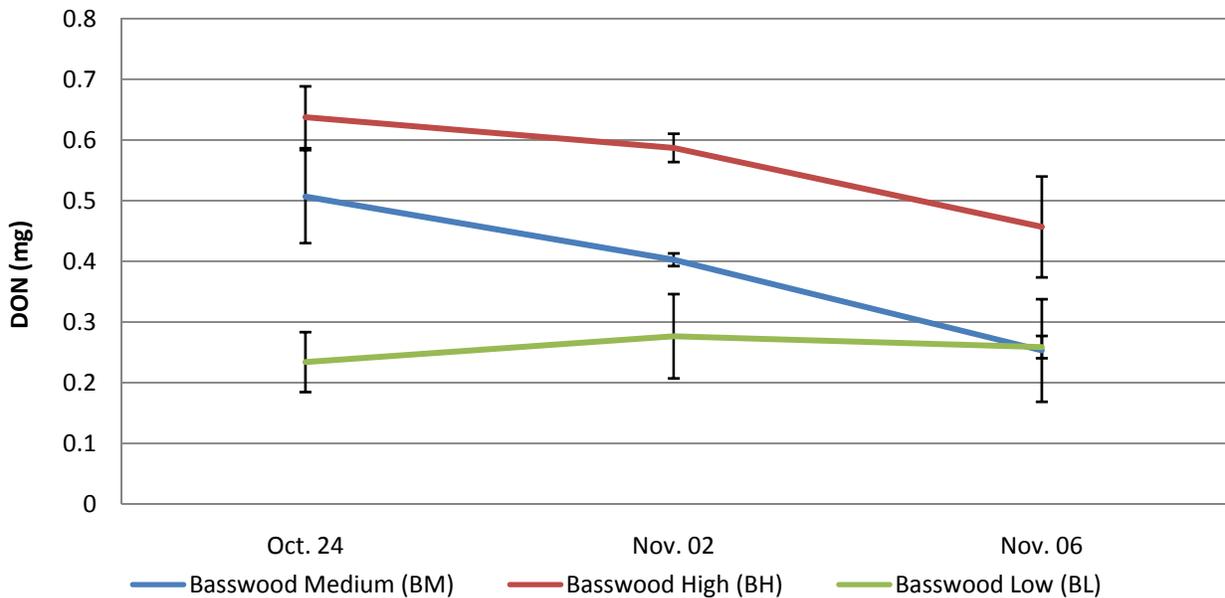
Figure 3.15: Dissolved organic nitrogen leached from basswood (B) leaf litter samples after treated with L (30mm), M (60mm) and H (100mm). The DON concentrations were corrected for treatment quantity and rainwater DON concentration.
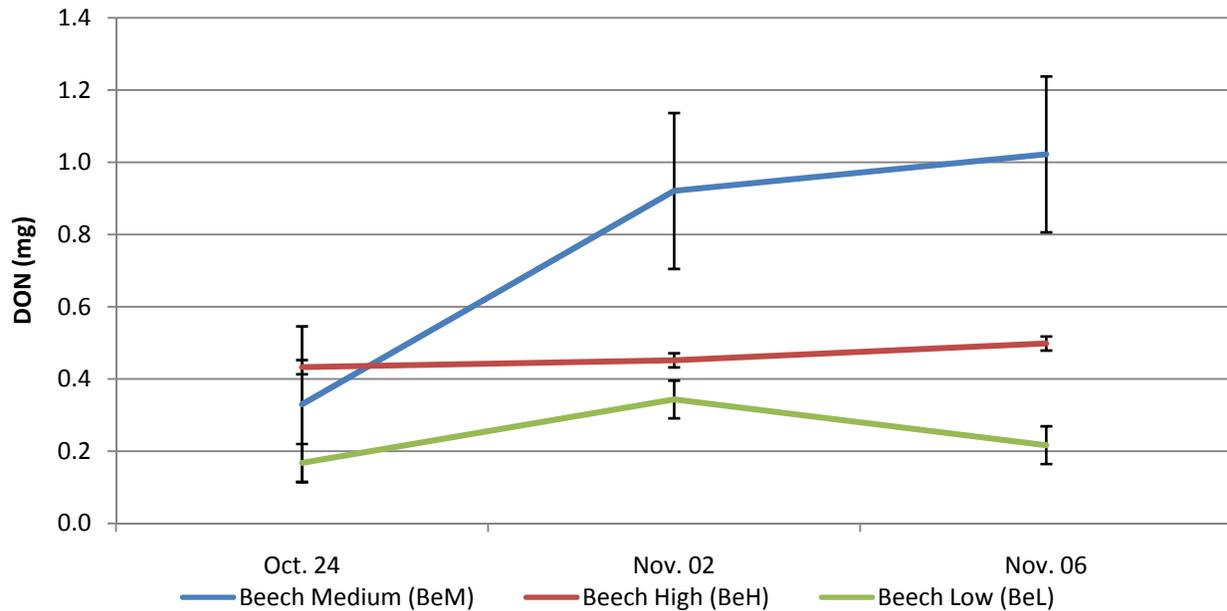
Figure 3.16: Dissolved organic nitrogen leached from beech (Be) leaf litter samples after treated with L (30mm), M (60mm) and H (100mm). The DON concentrations were corrected for treatment quantity and rainwater DON concentration.

The results of this study indicate that NH_4^+ and NO_3^- are both taken up by the plant litter in inconsistent concentrations throughout the two week period. In all treatments and litter types studied, NH_4^+ and NO_3^- are removed from the rainwater on the first day of treatment. Nitrate was more readily taken up by the plant litter throughout the study period. Sugar maple leaf litter continuously took up NO_3^- from the rainwater, in all treatments, throughout the entire length of the experiment (Figure 3.17). Although very similar, in most cases sugar maple L removed more NO_3^- from the rainwater, followed by sugar maple M and then sugar maple H. For basswood leaf litter, only those treated with L took up NO_3^- from the rainwater after the first day (Figure 3.18). Beech leaf litter did not remove any more NO_3^- from the rainwater after the first application until after day nine of the study, at which time all of the samples took up NO_3^- from the rainwater (Figure 3.17). With the exception of sugar maple and beech net NO_3^-, inorganic N leaching displayed a pattern. Leaching of inorganic N increased throughout the first 10 to 12 days, after which concentrations rapidly decreased. All three litter types generally leached similar concentrations of NO_3^- and NH_4^+. No consistent trends appeared, preventing a clear understanding of how the level of precipitation affects NO_3^- and NH_4^+ leaching. It should be

noted that this study assumed that if leachate concentrations of NO_3^- and NH_4^+ were higher than rainwater concentrations then the litter did not remove any NO_3^- and NH_4^+ from the rainwater.
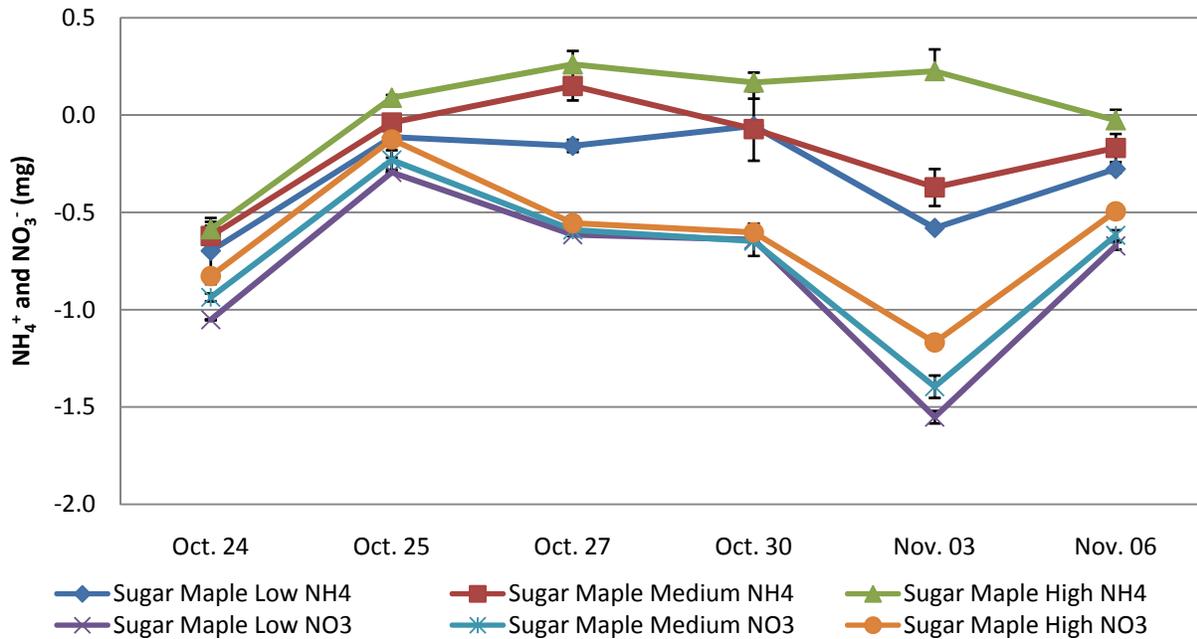
Figure 3.17: Dissolved inorganic nitrogen, measured as ammonium (NH_4^+) and nitrate (NO_3^-), leached from sugar maple (M) leaf litter samples after treated with L (30mm), M (60mm) and H (100mm). Ammonium and NO_3^- were corrected for treatment quantity and rainwater NH_4^+ and NO_3^- concentrations.
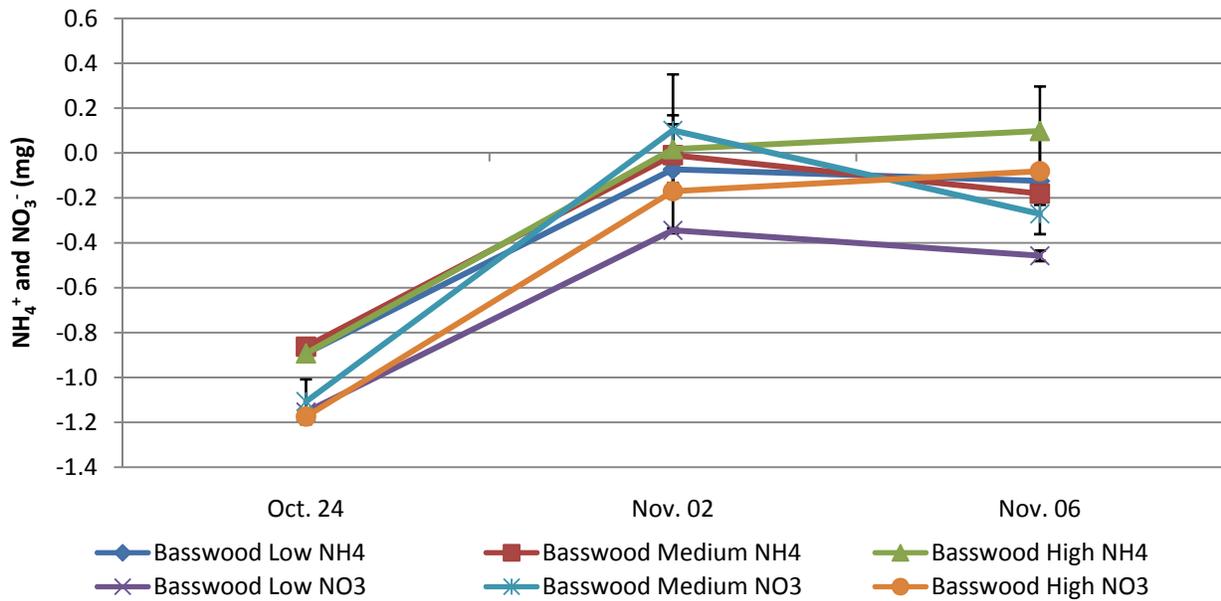
Figure 3.18: Dissolved inorganic nitrogen, measured as ammonium (NH_4^+) and nitrate (NO_3^-), leached from basswood (B) leaf litter samples after treated with L (30mm), M (60mm) and H (100mm). Ammonium and NO_3^- were corrected for treatment quantity and rainwater NH_4^+ and NO_3^- concentrations.
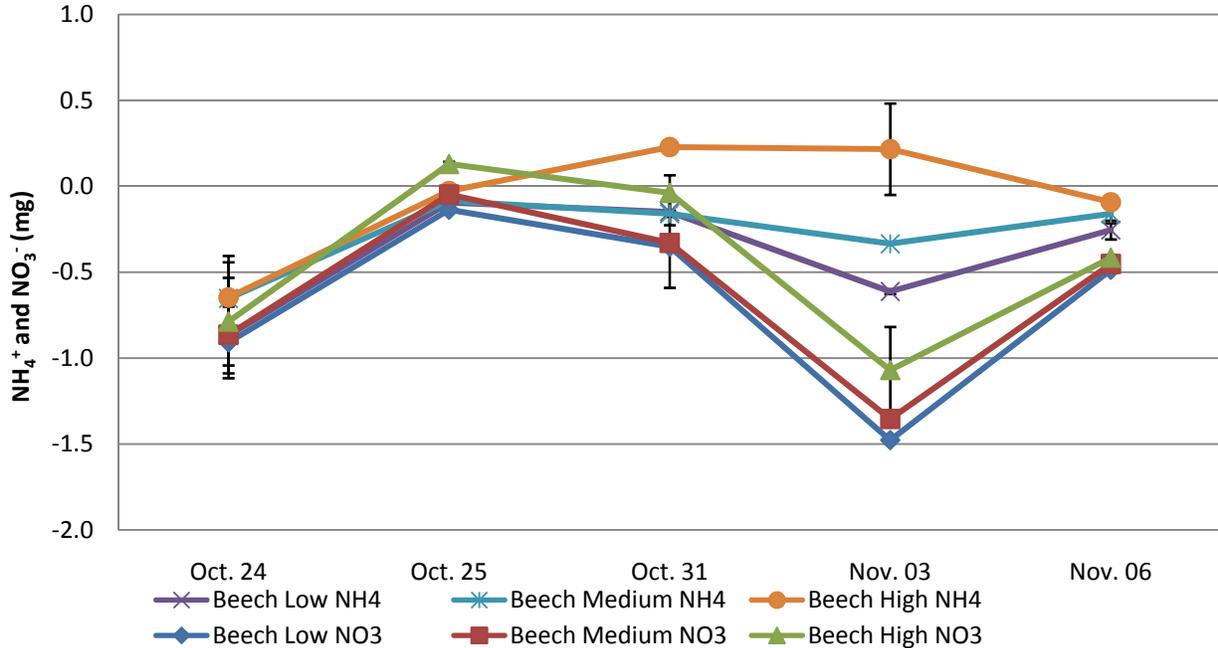
Figure 3.19: Dissolved inorganic nitrogen, measured as ammonium (NH_4^+) and nitrate (NO_3^-), leached from sugar maple (M) leaf litter samples after treated with L (30mm), M (60mm) and H (100mm). Ammonium and NO_3^- were corrected for treatment quantity and rainwater NH_4^+ and NO_3^- concentrations.

The up-take of inorganic N by leaves has been reported in many studies. Throughfall can be altered by becoming enriched by dry-deposited elements and leaching of plant material, or by loss of nutrients through absorption of ions from the incident rainfall (Potter et al., 1991; Gundersen et al., 2006). Specifically, many investigations into this topic have found that inorganic nitrogen (NH_4^+, NO_3^- and NO_2) is readily adsorbed from rainwater (Lovett and Lindberg, 1984; Yavitt and Fahey, 1986; Potter et al., 1991). This phenomenon has also been observed when rainwater (incident or throughfall) is in contact with leaf litter. Segal et al (1990) found that NH_4^+ concentrations in throughfall were lower than concentrations in leachate, suggesting that the litter absorbed NH_4^+. Nitrate, however, was more concentrated in the throughfall than in the leachate, suggesting that it had been leached from the litter, at least during the dormant season (Segal et al., 1990). Similar to this study, Ukonmaanaho and Starr (2001) evaluated the loss of nutrients from litter collecting in litter traps. Ukonmaanaho and Starr (2001) also found that N was taken up from the percolating throughfall.

3.6 Summary and Conclusion

The results of this study indicated that precipitation does affect sugar maple, basswood and beech leaf litter in litter traps for a two week period. The three litter types studied showed signs of leaching when exposed to medium precipitation (60mm, mean for the month of October), as well as below (30mm) and above medium (100mm). However, the influence of the precipitation was variable. Each species responded uniquely to the different treatments.

Precipitation induced a decrease in the dry weight in all species studied. Sugar maple exhibited the greatest decrease, followed by basswood and beech. For basswood and sugar maple, increased precipitation narrowed the C/N ratio, increasing the litter quality. The decreased C/N ratio is due to an increase of N. For beech, precipitation increased the C/N ratio of the leaf litter through initiating a loss of N. This study found basswood's pre and post experiment C/N ratio to be the lowest, followed by sugar maple, then beech. Potassium and Mg were readily lost from all litter. The loss of K is proportional to the precipitation magnitude. In the case of sugar maple, the loss of Mg was proportional to the precipitation applied. For basswood and beech, treatment H lost the greatest amount of Mg. Basswood lost more Mg in treatments with L

than M, and beech lost more Mg in treatments M than L. Phosphorus was not measured in any of the beech samples, was found to increase in all basswood samples, and increased in L and H treated sugar maple samples. The leaching of DOC was proportional to precipitation treatments for sugar maple and basswood samples. For beech, DOC leached most in treatment M, followed by H and L. Similarly, DON leaching increased with increased precipitation. In most cases the presence of DON was higher in the precipitation than in the leachate, suggesting it was taken up by the litter.

A relationship between leaching of C, N, P, K, Ca and Mg and the different precipitation regimes could not be made. Each species and tested parameter (C, N, P, K, Ca and Mg) responded differently. Based on a two week interval, precipitation generally induces a loss in easily leachable nutrients, like K. Elements associated with structural components, like C and Ca, remain relatively constant. Nutrients are likely to be assimilated from rainwater, especially N. Elements limiting microbial growth will be immobilized, like P, otherwise will be leached if they are readily lost.

Chapter 4

Freeze-Thaw Experiment

4.1 Introduction

Cycles of freeze-thaw accelerate litter decomposition, nutrient mineralization rates, nutrient leaching and gas fluxes from the soil (Campbell et al., 2005; Matzner and Borken, 2008). Measurable changes in C and N cycling and emissions of CO_2 and N_2O have been observed in forested ecosystems as a result of freeze-thaw cycles (Matzner and Borken, 2008). The changes induced by freeze-thaw are related to increased microbial activity and changes to the soil structure, as well as an increase in the death of roots (Campbell et al., 2005; Matzner and Borken, 2008).

It is believed that microbes are active during freeze and thaw events as evident from measurable levels of N_2O and CO_2 (Matzner and Borken, 2008). However, no consensus has been reached on the relationship between microbial activity and the presence of N_2O and CO_2. Some argue that freeze-thaw has no effect on microbial biomass (Lipson et al., 2000; Grogan et al., 2004). Instead it is the result of a build-up of gases in active unfrozen sites (Koponen et al., 2001; Matzner and Borken, 2008). Nitrous oxide and CO_2 are unable to release from soil due to decreased diffusion and advection until the soil thaws (Koponen et al., 2001; Matzner and Borken, 2008). This was typically found in studies involving alpine and tundra environments (Lipson et al., 2000; Grogan et al., 2004). In other cases, in particular arable soils, freeze-thaw was found to decrease the microbial biomass (Matzner and Borken, 2008). The increased activity was explained by microbial necromass (Herrmann and Witter, 2002). The increased availability of nutrients from the dead microbes initiated a quick recovery of microbial communities following a freeze (Hermann and Witter, 2002; Matzner and Borken, 2008).

Freeze-thaw cycles may also affect the soil structure by making soil aggregates, previously unavailable, accessible for mineralization, increasing microbial activity (Morkved et al., 2006; Matzner and Borken, 2008). Nitrate leaching from soils has been shown to increase following freeze-thaw cycles, as Boutin and Robitaille (1995) observed under sugar maple trees.

75

Harris and Safford (1996) found that freezing and thawing of leaf-litter led to an increase in the leaching of soluble C, when compared to samples not exposed to cycles of freezing-thawing.

With the increased frequency of freeze-thaw cycles follows a decrease in effects discussed previously. Specifically, a greater frequency of freeze-thaw cycles reduces microbial respiration and N mineralization rates (Harrmann and Witter, 2002). These are often greatest in the initial cycles and continually decrease with every subsequent freeze-thaw cycle (Herrmann and Witter, 2002). The extent to which freezing influences the process of decomposition is dependent on the species and intensity of freezing. After being thawed from very cold temperatures (-13 °C), soils of a northern hardwood-dominated forest (beneath sugar maple trees) showed an increase in CO_2 and N_2O and C and N cycling (Neilsen et al., 2001). Neilsen et al. (2001) also showed that freezing these soils increased NH_4^+ leaching, but decreased NO_3^- leaching. However, this is not typical, autotrophic nitrifiers are generally believed to be vulnerable to stress, like freezing, and recover slowly (Neilsen et al., 2001).

Although there have been a number of studies concerned with the effects of freeze-thaw on soil, much less attention has been paid to its effect on leaf-litter. However, similar to the influence of freeze-thaw on soil, leaves exposed to freeze-thaw are believed to become fragmented, leading to increased leaching and vulnerability to microbial deterioration (McBrayer and Cromack, 1980; Taylor and Parkinson, 1988e). Cycles of freeze-thaw are hypothesized to increase fragmentation (physical damage) of leaf-litter increasing the release of soluble compounds and subsequently increasing decomposition (Ivarson and Sowden, 1970; Skogland et al., 1988; Fitzhugh et al., 2001).

Taylor and Parkinson (1988e) conducted the first study to determine if repeated cycles of freeze-thaw accelerated the decomposition of aspen and pine leaf-litter. Results determined that freeze-thaw cycles can induce cellular structural damage to the leaf litter, specifically degrading the cuticle and allowing for increased water absorption (Taylor and Parkinson, 1988e). Consequently, this increased the leaching of substances from the leaf litter and increased microbial invasion (Taylor and Parkinson, 1988e). Taylor and Parkinson (1988e) concluded that water absorption was related to the frequency of freezing and thawing and not the length of time

76

the freezing occurred, however, repeated freezing of aspen leaves eventually no longer induced leaching. Similarly, Barlocher (1991) and Herrmann and Witter (2002) suggested that freshly abscised litter exposed to frost increased leaching losses. Many studies reported that cycles of freeze-thaw disrupt the physical state of the litter, by affecting the cell health, causing soluble components to be released (Barlocher, 1992; Harris and Safford, 1996). For leaf-litter, freeze-thaw cycles have varying affects depending on the species, moisture content of the litter, temperature fluctuations, the extent of freezing (lowest temperature reached), rate of temperature change, the frequency of freeze-thaw events and the duration of freezing and thawing periods (Taylor and Parkinson, 1988e).

4.2 Objectives

The objective of this study was to measure the change in dry mass and nutrient content of deciduous leaf litter, specifically sugar maple (M), basswood (B) and beech (Be), exposed to freeze-thaw temperatures. The intention was to evaluate the response of leaf litter to freeze-thaw, and determine if leaf litter from various deciduous tree species respond differently. Two approaches were used to evaluate the change in the nutrient dynamics of freshly abscised leaf litter: (i) mass loss and change in nutrient (C, N, P, K, Ca and Mg) content of leaf litter during a two week period; and (ii) the leaching of dissolved organic carbon (DOC) and nitrogen (DON) and dissolved inorganic nitrogen (DIN). It was hypothesized that cycles of freezing and thawing would result in increased nutrient leaching and mass loss, and that basswood and sugar maple would leach more nutrients than beech leaves.

4.3 Material and Methods

4.3.1 Experimental Set-up

To test the affects of freeze-thaw on leaf litter, an additional laboratory experiment, similar to the laboratory leaching experiment, was conducted. Leaf litter sampling and collection procedures are outlined in General Material and Method section (Chapter 1). Freshly abscised leaf litter, collected between October 22nd and 24th 2007, was used in the laboratory freeze-thaw experiment. Twenty grams (fresh weight) of leaf litter from each of the species, in triplicates,

were suspended over a plastic tub (0.32 x 0.27 x 0.12 m). Each treatment received 60 mm of water, simulating rainfall, over a 2-week period. On days 5, 9, and 13, the litter was frozen at 0°C for 3 hours. Simulated rainfall was applied daily at 9:00 am and litter was exposed to freezing temperatures from 16:00 hrs to 19:00 hrs. Rainwater collection and application procedure were the same as that outlined in Chapter 3. Control (Crtl) samples, in triplicates for each litter type, were also treated with 60 mm of rainfall over a two the week period but kept at room temperature throughout the study period. Leachate was collected on days following the freeze-thaw treatment to measure whether the freeze-thaw treatment induced greater leaching of the leaf litter (days 1, 6, 10 and 14).

4.3.2 Sample Analysis

After the 14-day study period, the litter was dried at 65 °C for 36 hours. The samples were ground in a Wiley Mill through a 2 mm sieve and subsequently ground with a Retschball mill. The samples were analyzed for phosphorus (P), potassium (K), Magnesium (Mg) and calcium (Ca) at the Soil and Nutrient Laboratory at the University of Guelph. Carbon (C) and nitrogen (N) analysis was conducted using a Costech Instrument ECS 4010 through elemental combustion analysis. Leachate was analyzed for ammonium (NH_4^+), nitrate (NO_3^-), and total Kjeldahl nitrogen (TKN) using colorimetric analysis (AAIII Bran-Luebbe) and Dissolved Organic Carbon (DOC) was analyzed using a Rosemont - Dohrmann High Temperature Total Carbon Analyzer.

4.4 Statistical Analysis

A one-way ANOVA was completed to compare the change in dry weight, C, N, P, K, Ca and Mg between treatments, as well as in comparison to the baseline (freshly abscised leaf litter collected on October 22nd and 24th 2007). All data sets were examined for homogeneity of variances between the two sets of compared data (using the Levene Test) and normal distribution was tested using Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test (Dytham, 2003). The variance of the data is homogeneous, normally distributed and the data is continuous. For all statistical analyses the threshold probability level for determining significant differences was $p<0.05$ and $p<0.10$.

To determine if there is a difference between treatment leachate concentrations over the study period repeated measures of ANOVA was completed. Repeated measures of ANOVA was completed to compare each of the measured parameters, DOC, DON, NO_3^- and NH_4^+, between treatments over the study period. Sampling time (over a two week period) was the repeated (within subjects) factor and DOC, DON, NH_4^+ or NO_3^- was the main (between subjects) factor. All data sets were examined for homogeneity of variances between the two sets of compared data (using the Levene Test) and normal distribution was tested using Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test (Dytham, 2003). The variance of the data is homogeneous, normally distributed and the data is continuous. Significance levels were adjusted using the Greenhouse-Geisser and Huyuh-Feldt to account for violations in sphericity.

4.5 Results and Discussion

4.5.1 Dry Weight Analysis

The leaf litter exposed to freezing and thawing exhibited a relative reductions in weight when compared to the control samples (litter not exposed to freeze-thaw conditions), with the exception of beech. Basswood leaf litter exposed to freeze-thaw cycles exhibited a significant ($p<0.10$) change in weight in comparison to basswood control. Sugar maple leaf litter did not significantly decrease but a visible decline was produced. Beech leaf litter, on the other hand, displayed higher dry weight results then the control.

It was hypothesized that there would be a greater decrease in weight among the treated samples compared to the control samples. This is mainly attributed to increased leaching as a result of structural disruption and membrane rupture (Tukey 1966; Burke et al., 1976; Hurst et al., 1985), producing a greater loss in mass (Swift et al., 1981). Therefore, it is no surprise that treated basswood and sugar maple leaf litter exhibited a greater loss in weight. Many studies have reported a decrease in mass over winter months (Gosz et al., 1973, Lousier and Parkinson, 1976; Hobbie and Chapin, 1996). The loss was concluded to be due to physical processes, like fragmentation and leaching associated with freeze-thaw (or biologically activity during the winter). Barlocher (1992) found that sugar maple leaf litter loss in mass was greater when

79

exposed to freeze-thaw conditions. Yet, some studies have reported that freeze-thaw has no significant affect on senescent leaf litter (Hurst et al., 1985; Melick and Seppelt, 1992). Melick and Seppelt (1992) reported that a lack of significance implies that since the action of freeze-thaw alters the membrane, senescent leaf litter is not greatly affected as the membrane has already undergone rupturing. The apparent lack of response from beech leaf litter in this study follows Melick and Seppelt (1992) and Hurst et al. (1985) findings. Furthermore, a greater loss in mass of the this study's control suggests that cycles of freeze-thaw may have inhibited normal microbial and leaching activity. Studies have found that microbial communities can tolerate slow freezing and thawing cycles, similar to what would occur in the field (Campbell et al., 2005). This experiment induced a rapid change in temperature that may have negatively affected the microbial communities.

Table 4.1: Mean dry weight (g) of control (samples exposed to 60 mm of precipitation) and freeze-thaw (samples exposed to 60 mm of precipitation and freeze temperatures 3/bi-weekly) treated sugar maple, basswood and beech leaf litter. Initial weight refers to the dry weight of leaf litter prior to the experiment and after refers to the dry weight post- 14-day study period. Standard error is given in parenthesis.

	Control		Freeze-thaw	
Species	Initial (g)	After (g)	Initial (g)	After (g)
Sugar Maple	11.33	10.52(0.59)	11.33	10.05(0.27)
Basswood	12.19	14.66(0.19)	12.19	13.87(0.22)
Beech	11.21	9.77(0.31)	11.21	13.87(0.38)
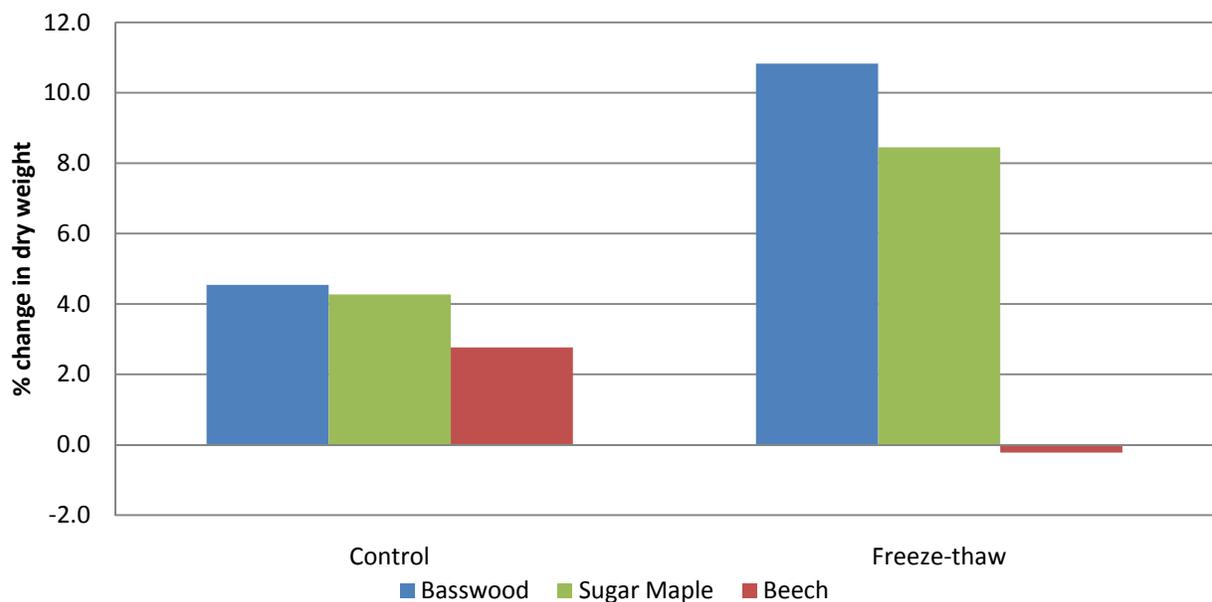
Figure 4.1: Mean percent change in dry weight (comparison of the initial to the final dry weight) of control (60 mm of precipitation) and freeze-thaw (60mm of precipitation and freeze-thaw) treated sugar maple, basswood and beech. Positive values identify a decrease in the weight upon completion of the experiment and a negative value identifies an increase.

4.5.2 Nutrient Analysis

Cycles of freeze-thaw accelerate litter decomposition and nutrient mineralization rates (Campbell et al., 2005; Matzner and Borken, 2008). Freeze-thaw cycling affected the elemental content of the leaf litter, however, each litter type responded differently. Significant and measurable changes to C and N cycling have been observed in forested ecosystems (Matzner and Borken, 2008). This study's basswood sample results corroborate these findings. Basswood exhibited a decrease in the C/N ratio in the control sample but a larger decrease in the treated samples (Figure 4.2). The decrease in the C/N ratio within the control is due to an increase in N, similar to Chapter 3 results, likely due to the assimilation of N from the applied rainwater. In the treated basswood samples, the decrease in C/N ratio is a result of decreased C content. This may be a result of leaching due to increased fragmentation of leaf litter (McBrayer and Cromack, 1980).

Sugar maple and beech samples exposed to freezing and thawing conditions did not demonstrate a decrease in C/N ratio. Sugar maple control samples had an increase in N, resulting in an increase in C/N ratio, similar to Chapter 3's findings. The treated sugar maple sample's C and N content was very similar to freshly collected leaf litter, suggesting that the C and N content was not affected by freezing and thawing. Both the control and treated beech samples exhibited an increase in the C/N ratio as a result of a decrease in N and no change in the C concentration. Beech control sample lost less N than the treated sample and thus had a greater C/N ratio than the beech treated samples (Table 4.2).
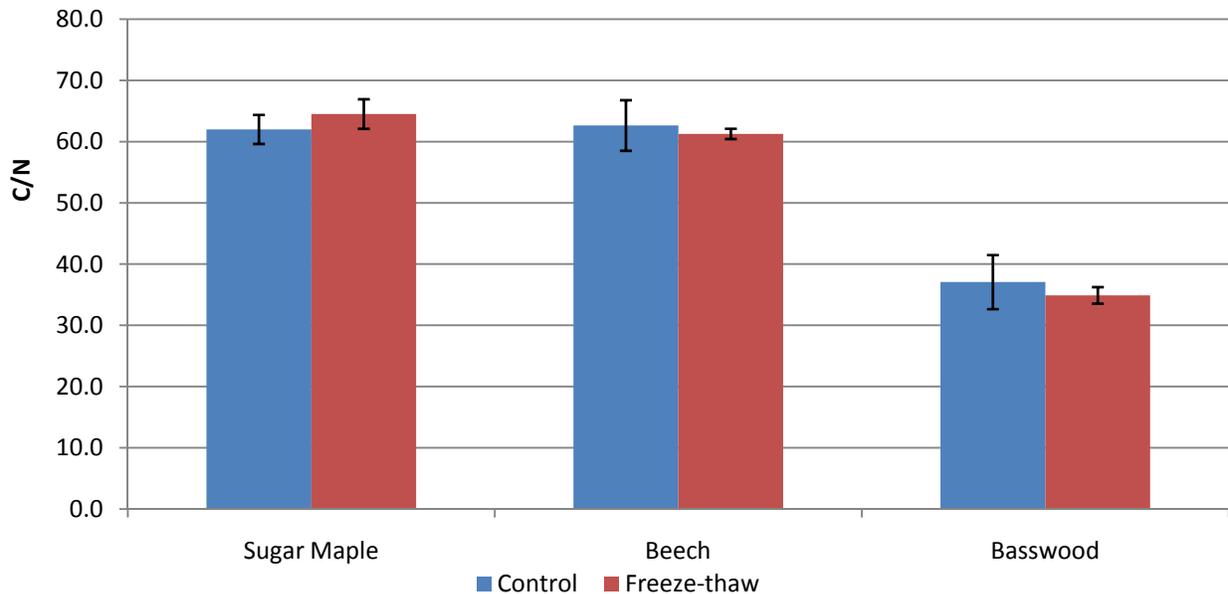
Figure 4.2: Mean carbon to nitrogen (C/N) ratio of control and freeze-thaw treated sugar maple, basswood and beech leaf litter after the 14-day freeze-thaw experiment.

Similar to Chapter 3's results, the potassium (K) and phosphorus (P) content in sugar maple and beech litter fell below the detectable limit in both the control and treated samples (Figure 4.3). For K this is <0.30 mg/kg and for P this is <0.05 mg/kg. Freshly abscised sugar maple and beech litter had measurable levels of K, 0.42 mg/kg and 0.31mg/kg, respectively, and sugar maple had P concentration of 0.57 mg/kg (Table 4.2). This suggests that freeze-thaw did not inhibit the leaching of K and P from sugar maple and beech. As previously stated, K is readily leached from litter (Hagen-thorn et al., 2006). Therefore, it is possible that after the first

precipitation application the majority of K was lost from the litter. If this is the case, cycles of freeze-thaw would not have had an opportunity to affect K leaching.

Both K and P have been shown to leach during the initial phase of decomposition (Waring and Schlesinger, 1985; Hagen-Thorn et al., 2006; Gosz et al., 1973; Hurst et al., 1985; Kwabaih et al., 2001; Tukey). Potassium concentration of cells near the surface of the leaf makes its leaching predictable (Waring and Schlesinger, 1985). Potassium is not dependent on mineralization for release from leaf litter (Cole and Rapp, 1981) and is generally not limiting for microbial growth (Waring and Schlesinger, 1985). Phosphorus is not as predictable, and is more dependent on the requirement for growth of decomposers (Swift et al., 1979). Although many have found P to leach during early stages of decomposition, it is also readily immobilized (Waring and Schlesinger, 1985). Immobilization of P either follows the initial leaching phase (Kwabaih et al., 2001) or immediately, depending on the requirement of P for microbial growth (Swift et al., 1979). As well, P and K are both retranslocated from the leaf back to the plant, but this process is much more pronounced in P (Waring and Schlesinger, 1985). Freeze-thaw was found to decrease the K content of basswood leaf litter. As mentioned, this is most likely due to the readily leachable nature of K (Gosz et al., 1973). Hurst et al. (1985) found that cycles of freeze-thaw accelerated the loss of K from litter. The differences in K concentration between sugar maple, basswood and beech, in this study, are likely due to differences in the initial K concentration of the leaf litter prior to the study.

Basswood leaf litter exposed to freeze-thaw conditions had higher levels of P than the control samples, but lower than freshly abscised leaf litter. Phosphorus is known to leach from litter in early stages of decomposition (Kwabaih et al., 2001). From this two conclusions are formed, either treating the litter to repeated cycles of freeze-thaw slowed down the loss of P or P did not leach from the litter. The former conclusion does not agree with the literature on the effect of freeze-thaw treatment on leaf litter. The literature states that cycles of freeze-thaw accelerate losses of nutrients through increased leaching (Campbell et al., 2005; Matzner and Borken, 2008). However, this may confirm Melick and Seppelt (1992) and Hurst et al. (1985) speculation as to the lack of effect of freeze-thaw on senescent litter. However, other parameters,

such as dry weight, suggest that freeze-thaw does accelerate senescent basswood leaf litter decomposition.

The latter conclusion is based on the high standard deviation of P in the freshly abscised leaf litter. The levels of P found in the freshly abscised samples were lower than the levels found in the control and freeze-thaw treatments. Variation in the concentration of elements can be due to the timing and duration of leaf fall from deciduous trees (Carlisle et al., 1966; Gosz et al., 1972). The climatic conditions at the time of senescence can facilitate leaf fall prematurely, thus leaf litter may have higher concentration than would otherwise if it had fallen naturally (Gosz et al., 1972). The variation in timing in leaf fall can have substantial ramifications on the litter quality and nutrient cycling (Taylor and Parkinson, 1988b). As well, P may have been immobilized by microbial activity, thus exhibiting a tiny increase in relation to the losses discussed earlier.

The Ca content of sugar maple litter exposed to freeze-thaw treatment decreased in comparison to the control (Figure 4.3), as well as decreased in comparison to freshly collected leaf litter (Table 4.2). This coincides with the conclusions of Tukey (1966), Burke et al. (1976) and Hurst et al. (1985) that freezing and thawing litter ruptures cellular structural components. Calcium is a part of this process and is often immobilized until structural decomposition. In comparison to freshly collected litter, Basswood leaf litter display a decrease in Ca, however, basswood control lost more Ca than treated litter. The Ca content of beech litter increased in both the control and freeze-thaw treated litter in comparison to freshly collected litter. The increase in Ca was greater in freeze-thaw treated beech litter than the control litter.

The loss of Ca is dependent on the decomposition of litter as it is an immobile element (Likens et al., 1998). Since freeze-thaw cycles instigate a faster initial rate of decomposition by increasing the leaching of leaf material (Tukey 1966; Burke et al., 1976; Hurst et al., 1985), freeze-thaw should increase the loss of Ca. The findings reported for sugar maple litter follow this rationale. Beech leaf litter did not exhibit a decrease in weight, suggesting that it was unaffected by cycles of freeze-thaw. For beech, the Ca content was proportionate to the weight such that the control exhibited both a greater decline in weight and Ca, than the treated litter. The

lack of Ca lost from beech leaf litter further suggests that cycles of freeze-thaw do not contribute to the decomposition of beech litter. As stated previously, different results may have been found had the temperature gradually changed. This may also explain why treated basswood litter lost less Ca than the control samples.

Exposing sugar maple and basswood leaf litter to freeze-thaw conditions produced a greater loss of magnesium (Mg) relative to the control sample (Figure 4.3). These finding are very similar to K results as was expected. Like Potassium, Mg is a readily leachable element (Gosz et al., 1973; Tukey 1970). These results suggest that freeze-thaw accelerates the leaching in both sugar maple and basswood. These finding corroborate those of Barlocher (1991), Melick and Seppelt (1992), Harris and Safford (1996) and many more, all of whom find that freeze-thaw accelerated the leaching of material from leaf litter.

For beech leaf litter, the opposite was found with regard to Mg (Figure 4.3). Treated beech leaf litter, although exhibiting a decrease in Mg concentrations in comparison to freshly collected litter, showed less of a decrease in Mg than the control. This result follows all of the trends regarding beech nutrient concentrations reported previously. This reiterates the likelihood of either a lack of freeze-thaw affect or experimental error.
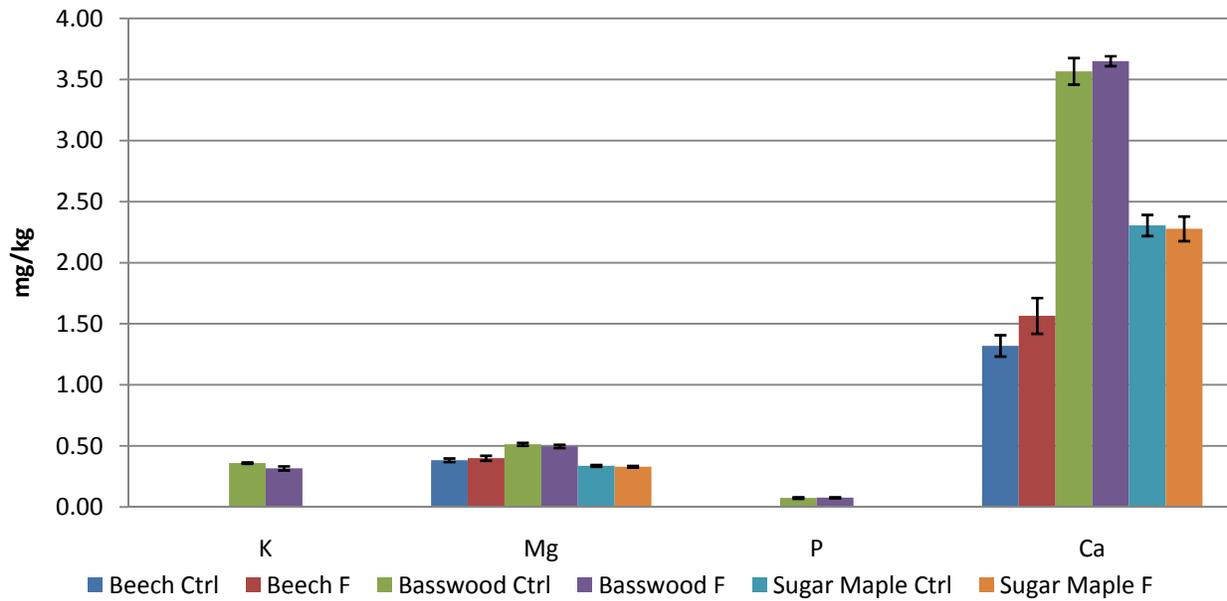
Figure 4.3: Mean nutrient content (mg/kg) of control (60 mm of precipitation) and freeze-thaw (60mm of precipitation and freeze-thaw) treated sugar maple (M), basswood (B) and beech (Be) leaf litter. Control treatment is abbreviated to Ctrl and freeze-thaw to F.
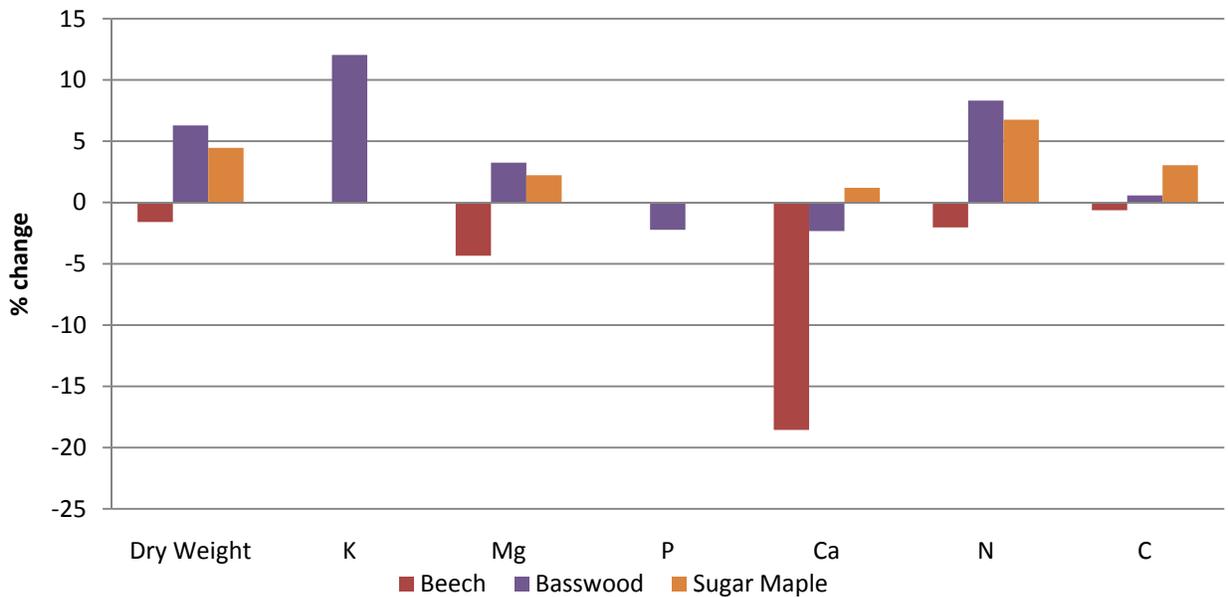
Figure 4.4: Percent change (comparison of the control and post- 14-day experiment) of nutrient content (mg/kg) in freeze-thaw treated sugar maple (M), basswood (B) and beech (Be) leaf litter in comparison to the control. A negative percent change identifies an increase while a positive percent change identifies a decrease.

4.5.3 Leachate Analysis

The concentration of DON, DON, NH_4^+ and NO_3^- in the leachate (rainwater percolating through the leaf litter) were corrected for rainwater DOC, DON, NH_4^+ and NO_3^- concentrations. Measured concentrations of DOC, DON, NH_4^+ and NO_3^- were corrected for the quantity of treatment applied. This was done to correct for a dilution effect. Reported negative values identify when rainwater nutrients were taken up by the leaf litter resulting in a lower concentration in the leachate.

After the initial treatment, the loss of DOC from beech leaf litter exposed to cycles of freeze-thaw was greater than the beech control samples (Figure 4.5). Similarly, Harris and Safford (1996) found that freeze-thaw significantly increased the loss of soluble C from freshly abscised leaf litter. Melick and Seppelt (1992) and Hurst (1985) found that (pre-senescent) litter exposed to freeze-thaw cycles leached more carbohydrates. Contrary to these findings, the sugar maple and basswood control samples leached more DOC than was lost from leaf litter exposed to cycles of freeze thaw (Figure 4.5). As previously mentioned, some studies have found that senesced leaf litter is not significantly affected by freeze-thaw cycles (Melick and Seppelt, 1992; Hurst, 1985). Schimel and Clein (1996) found a large flux of CO_2 from soil following exposure to freeze-thaw. This was attributed to an increase in metabolic activity in response to increased organic substances from microbial death (Schimel and Clein, 1996). The results of this study indicate that increased microbial metabolism may reduce the loss of organic matter from the plant material. Matzner and Borken (2008) suggest that physical disruption of aggregates by frost might cause increased bursts of microbial activity as a result of increasing available food sources. Taylor and Parkinson (1988e) found that the leaching from aspen leaf litter exposed to cycles of freeze-thaw was less then litter not exposed to freezing and thawing temperatures. However, Taylor and Parkinson (1988e) found that pine needles leached more when treated with freeze-thaw cycles. The lack of leaching by aspen litter suggested that the water soluble content of aspen leaves was rendered insoluble by repeated freezing (Taylor and Parkinson, 1988e). The results from Taylor and Parkinson (1988e) determined that the cuticle is damaged during repeated freeze-thaw applications. The damaged cuticle allows microbial organism's access to easily decomposable matter that would normally be protected (Taylor and Parkinson, 1988e).
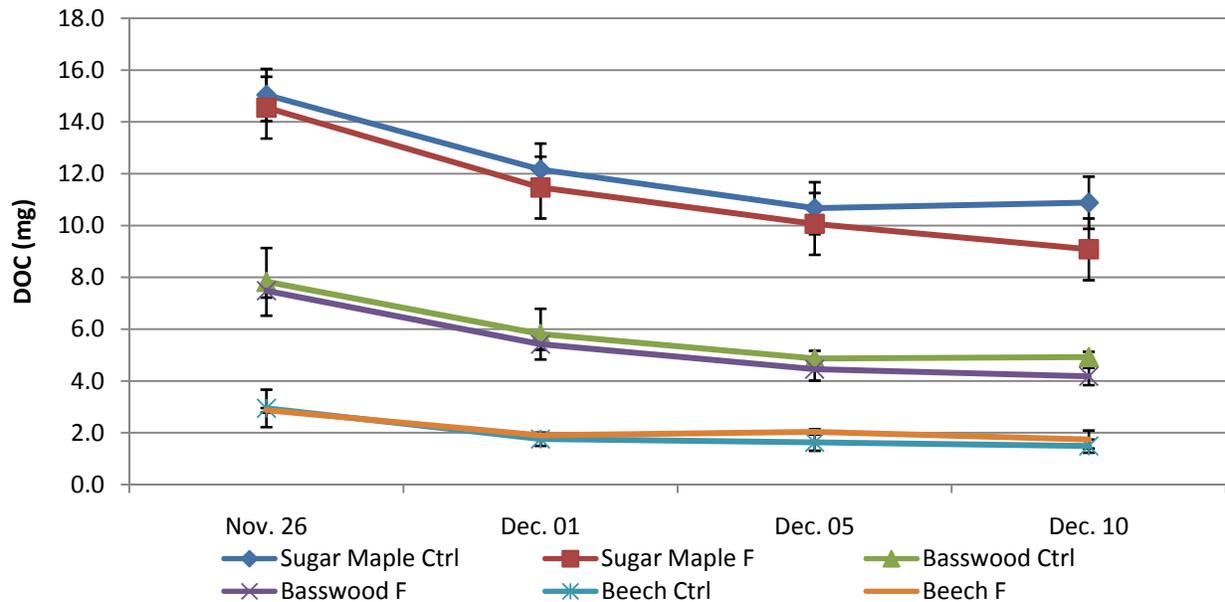
Figure 4.5: Dissolved organic carbon (DOC) measured in mg leached from control (Ctrl) and treated (F) sugar maple, basswood and beech leaf litter over the two week study period.

The pattern of DON leaching resembled DOC leaching (Figure 4.6). Specifically, the loss of DON from beech litter exposed to cycles of freeze-thaw were higher than the control. Basswood leaf litter exposed to freeze-thaw treatment leached less DON then the control. Leaching of DON from sugar maple behaved slightly different. On day 10, sugar maple leaf litter exposed to freeze-thaw treatment leached more DON then the control sample. As expected, DON measured on the other days was lower in treated samples than control samples.

In all three litter types DOC was leached in greatest quantities on the first day. Both sugar maple and basswood DOC levels declined over the two week period. Beech leaf litter also exhibited a decline in DOC leaching, although not to the same extent. For DON leaching, basswood was the only litter type to leach the greatest quantity on the first day and then show a continual decrease of the remainder of the study. Both beech and sugar maple lost the lowest level of DON on the first day. From there, beech leaf litter displayed a plateau. Similar to DOC, sugar maple litter exposed to freeze-thaw had decreased DON concentrations by the end of the

experiment, while the control continually increased. Furthermore, both control and treated, sugar maple leaf litter leached more DOC than basswood, while basswood leached more DOC than beech (Sugar Maple>Basswood>Beech). The concentration of DON fluxes where similar throughout the three litter types.
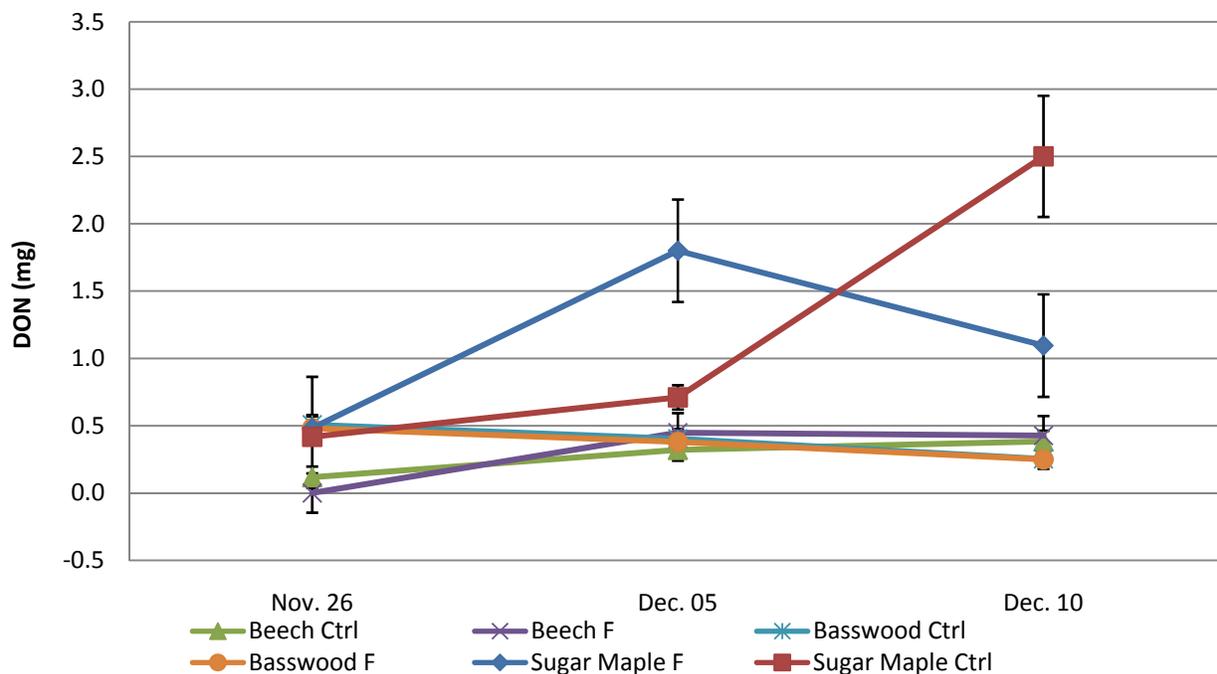
Figure 4.6: Dissolved organic nitrogen (DON) measured in mg leached from control (Ctrl) and treated (F) sugar maple, basswood and beech leaf litter over the two week study period.

In all cases, NH_4^+ and NO_3^- were assimilated from the rainwater on the first day of the experiment. The remainder of the sampling period indicated that NH_4^+ was lost from all three litter types. Analysis of the leaching pattern found that exposing litter to freeze-thaw cycles delayed the loss of NH_4^+. While treated samples continued to leach NH_4^+, peaks exhibited in the control where displayed in the treated samples later in the study. By the end of the study NH_4^+ levels had begun to decline in all control samples, as well as in sugar maple and beech treated samples (Figures 4.7 and 4.9). Basswood samples exposed to freeze-thaw conditions showed an increase in NH_4^+ leaching compared to earlier concentrations and to the control (Figure 4.8). However, the increase displayed by basswood litter may be a lag affect induced by NH_4^+. It is

believed that the concentration would have again decreased if measured after day 14. Neilson et al. (2001) found that soils under areas dominated by sugar maple exhibited an increase in NH_4^+ and a decrease in NO_3^- leaching, along with increased N mineralization. Although, in this study sugar maple did not display increased NH_4^+ and decreased NO_3^- in comparison to the control, overall NH_4^+ was leached while NO_3^- was not.

Throughout the two week freeze-thaw period, the majority of the sampling days found that NO_3^- was taken up by treated litter. This is in contrast to the often reported increase in NO_3^- leaching from forest ecosystems following a frost (Matzner and Borken, 2008). Both beech and sugar maple litter exposed to freeze-thaw cycles exhibited a loss of NO_3^- at the beginning of the study (Figures 4.7 and 4.9). This may indicate that litter exposed to freeze-thaw can induce a loss of NO_3^-, rather than absorption from the rainwater. However, this is not substantiated by concentrations found after this initial period. In basswood, treated samples did not leach NO_3^- while control samples did. This would indicate that freeze-thaw cycles inhibit leaching of NO_3^-, and promote absorption of NO_3^- from rainwater. While Hobbie and Chapin (1996) found that N was lost from litter during the winter months, due to biological or physical processes related to freezing and thawing, the leaching of NH_4^+ and NO_3^- did not increase.

Overall, treated litter lost less NH_4^+ and NO_3^- than the control samples. However, following the freezing periods NH_4^+ and NO_3^- showed relative increases in the loss of inorganic N. Schmidt et al. (2007) and Schimel and Clein (1996) found similar results, although their studies took place using soil. Schmidt et al. (2007) found that microbial turnover is a substantial source, as well as a sink, for DON and DIN following winter thaw in soils. Schimel and Clein (1996) found that freeze-thaw cycles caused a flush of microbial N, and that increased exposure minimized the ability for microbial communities to decompose SOM.
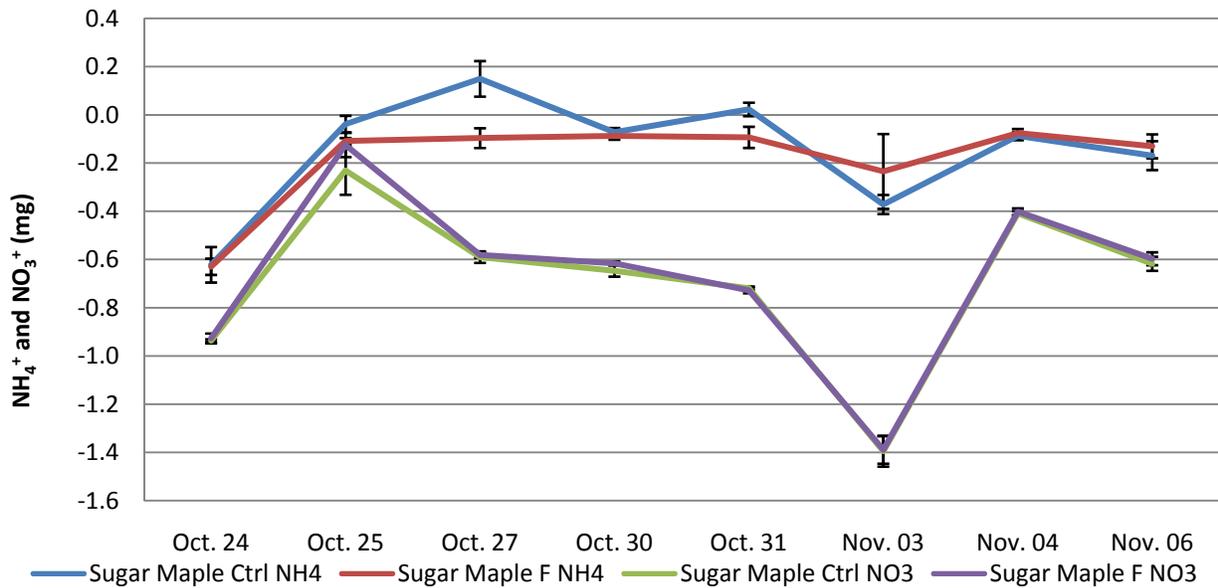
Figure 4.7: Dissolved inorganic nitrogen (NO_3^- and NH_4^+) measured in ppm leached from control and treated sugar maple over the two week study period. Negative values identify an assimilation of inorganic N by the leaf litter.
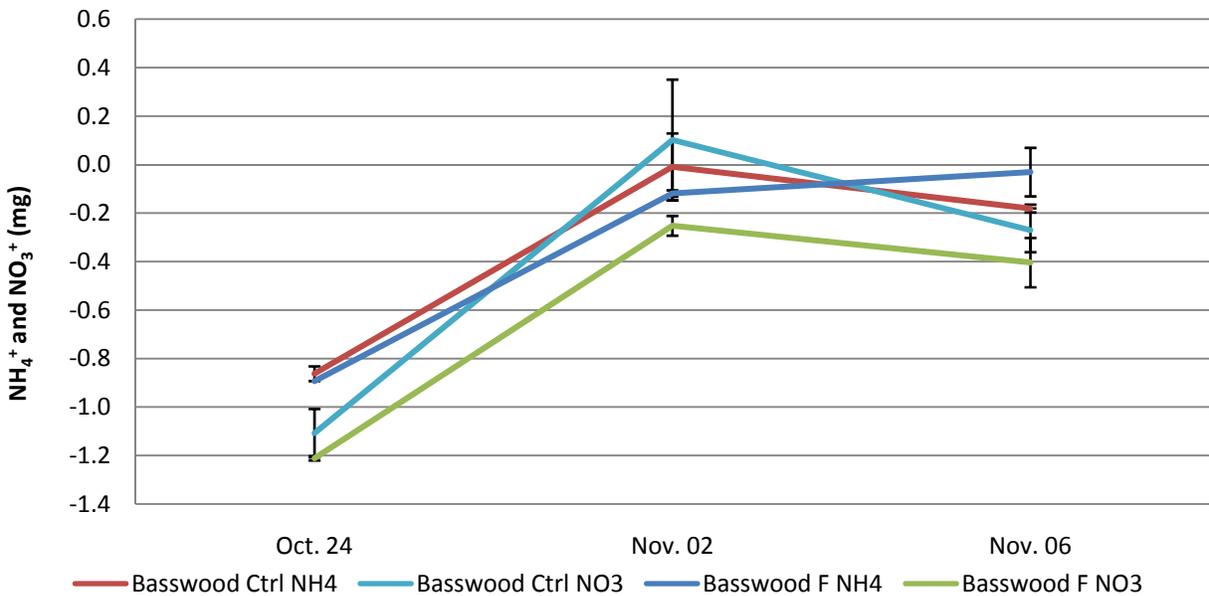
Figure 4.8: Dissolved inorganic nitrogen (NO_3^- and NH_4^+) measured in mg leached from control and treated basswood over the two week study period. Negative values identify an assimilation of inorganic N by the leaf litter.

91
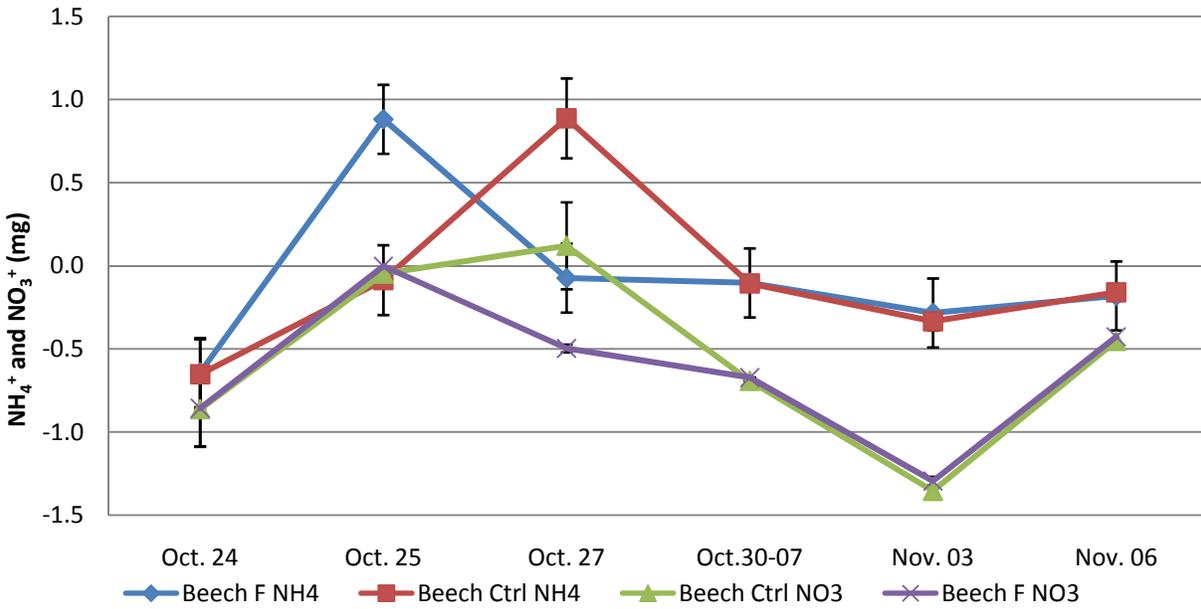
Figure 4.9: Dissolved inorganic nitrogen (NO_3^- and NH_4^+) measured in mg leached from control (Ctrl) and treated (F) beech over the two week study period. Negative values identify an assimilation of inorganic N by the leaf litter.

It is possible that the lack of significant findings in the study may be due to the methodological approach. As stated earlier, the leaves were exposed to cycles of freezing and thawing mid-way between applications. Taylor and Parkinson (1988e) found that cycles of freeze-thaw induced a greater mass loss when the moisture content was greater. This study did not take moisture into consideration. As well, the rate of temperature change may have influenced the results. Both Campbell et al. (2005) and Taylor and Parkinson (1988e) stated that microbial communities can tolerate slow freezing and thawing cycles, similar to what would occur in the field. This study induced a quick temperature change, from a 21˚C room to a 0˚C cooler.

The difficulty in predicting the affect of freeze-thaw on leaf litter is the number of variables that must be taken into account. As previously mentioned, Taylor and Parkinson (1988e) noted a number of factors to consider including species, moisture content, temperature range (specifically the final low temperature), rate of temperature change, duration of freezing and thawing periods, and the number of repeated cycles.

4.6 Summary and Conclusions

The results of this study indicate that freezing and thawing temperatures influence sugar maple, basswood and beech leaf litter in litter traps. Freezing and thawing leaf litter generally stimulated a greater decrease in weight in comparison to the control samples (litter not exposed to freeze-thaw conditions), with the exception of beech. Similarly, the litter quality (C/N ratio) was found to increase in sugar maple and basswood samples due to the leaching of N. Beech leaf litter exhibited a decrease in the C/N ratio as a results of increased N. Beech leaf litter exhibited an increase in N, Ca and Mg. Sugar mapleand basswood leaf litter displayed a decrease in N, Ca and Mg. Potassium was found to readily leach from all litter types. Phosphorus was measured in only basswood leaf litter and was found to increase. The leaching of dissolved organic carbon (DOC) and nitrogen (DON) was not influenced by freeze-thaw treatment. The leaching of DIN showed variable response. Beech leaf litter leached more NH_4^+ and NO_3^- when exposed to freeze-thaw temperatures, however, sugar maple and basswood did not.

Although this study has clearly identified that leaf litter in litter traps was influence by freeze-thaw treatments, determining a specific response was found to be unfeasible. The three deciduous species and the parameters measured responded differently. No identifiable trend could be determined to approximate changes in future studies. The differing responses may be related to different microbial communities and there response to freeze-thaw temperatures. Further investigation into the influence of freeze-thaw on phyllosphere (leaf surface) microbial community structure is required.

Chapter 5
Phyllosphere Microbial Experiment

5.1 Introduction

For over a century it has been well recognized that bacteria inhabiting the leaf's surface are distinct from bacteria that inhabit the pedosphere (Beattie and Lindow, 2003). The phyllosphere microbial community structure is influenced by leaf age, light incidence, and microclimate (Yadav, 2005).

The leaf surface, also commonly known as the phyllosphere, is considered an extreme environment as it is subject to severe environmental conditions (Hirano and Upper 2000; Yang et al., 2001; Lindow and Brandl, 2003). During the day, leaves are dry and exposed to high temperatures and intense UV radiation, where as in the night leaves are exposed to cool temperatures and are usually moist from dew (Andrews and Harris, 2000; Yang et al., 2001). However, this becomes a hostile environment due to the frequent rapid and repeatedly fluctuating conditions over short periods of time (Hirano and Upper 2000; Leveau, 2006). In a temperate environment, this is compounded by the changing physical and biological properties of the leaves over a season, such that deciduous leaves emerge, develop and senesce over just several weeks (Hirano and Upper 2000). Along with the leaf's surface topography, nutrient availability of the phyllosphere is an important regulator of the inhabiting microbial community (Ibekwe, 2000). In general the phyllosphere is nutrient deficient and is susceptible to damage (weathering) from wind and rain (Andrews and Harris, 2000; Yang et al., 2001).

However, it is well recognized that the phyllosphere supports an abundant and dynamic epiphytic microbial population (Jackson et al., 2006). It is considered one of the most commonly inhabited environments by terrestrial microorganisms (Lambias et al., 2006). This is likely due to the abundance of leaf surfaces available for colonization; estimated to be an area of 6.4×10^8 km^2 (Lindow and Brandl, 2003). Although bacteria, of many different genera, are the most abundant inhabitant of the phyllosphere, a diverse arrangement of microorganisms are also present

including filamentous fungi, yeast, algae, and to a much lesser extent protozoa and nematodes (Lindow and Brandl, 2003).

Studies that have conducted leaf-imprints demonstrate that bacteria colonize specific sites, and are not distributed uniformly (Beattie and Lindow, 2003). It is predominately found that epiphytic microbes colonize the phyllosphere in protective topographical areas, such as veins, trichomes, stomata openings, epidermal cell joint and depressions in the cuticle (Andrews and Harris, 2000; Beattie and Lindow, 2003). It has also been found that microbial populations tend to be greater on the underside of the leaf (abaxial), as opposed to the upper side (adaxial), likely due to better nutrient and microhabitat conditions (Andrews and Harris, 2000). Population size and location may fluctuate in relation to deposition of nutrients and the leaching of nutrients in order to optimally utilize the energy source. Leaves in the lower section of the canopy also tend to have a higher population of microbes (Andrews and Harris, 2000; Yadav et al., 2005), that may be due to increased protection from environmental stresses and greater opportunity to utilize nutrient deposition from throughfall. Microclimatic factors contribute to variations in the distribution of microbes over the leaf surface. For example temperature may differ from the centre of the leaf to the edge which will affect the rate at which water is evaporated from the surface and the availability of water to the microbes (Leveau, 2006).

Epiphytic populations vary considerably over the short and long term. This is mostly related to the fluctuations in the physical and chemical conditions typical of the phyllosphere (Lindow and Brandl, 2003). Short term fluctuations are generally influenced by environmental conditions, like rainfall, whereas long term fluctuations are prominently related to seasonal changes (Andrews and Harris, 2000). For example, Thompson et al. (1993) reported discrete colonization patterns throughout the year, cooler and rainy seasons supported the highest bacterial populations and in warm, dry seasons populations were greatly diminished. As well, younger leaves support the greatest diversity, as opposed to older leaves (Thompson et al., 1993). Bacterial abundance and metabolic activity are highest on moist leaves (Jackson et al., 2006). It is likely that bacterial populations reside in more protected areas, but take advantage of less inhabited areas when environmental conditions are favourable (Andrews and Harris, 2000).

Epiphytic bacteria present in the phyllosphere are believed to migrate from the surrounding air once the leaf has materialized (Kadivar and Stapleton, 2003). Suggested mechanisms for migration from the surrounding air include, deposition by aerosol, wind, rain, insects, and leaf-to-leaf transfer (throughfall) (Leveau, 2006; Lindow, 1996; Lilly et al., 1997; Andrews and Harris, 2000).

Because the phyllosphere is a nutrient limited environment (Ibekwe, 2000; Lindow and Brandl, 2003), the availability of carbon containing compounds is a significant limiting condition for the colonization microorganisms (Lindow and Brandl, 2003). The most abundant source of nutrients available to support microbial communities is plant metabolites, like photosynthates, specifically, glucose, fructose and sucrose (Leveau, 2004). These become most readily available through leaching (Leveau, 2006). This corroborates, the general understanding that microbial communities thrive, or are most plentiful, when the temperature is cool and the conditions are moist, coincidently conditions which promote leaching. The composition and quantity of nutrients, like carbohydrates, organic acids and amino acids, that sustain the epiphytic community are influenced by plant species, leaf age, leaf physiological status and the presence of tissue damage (Yang et al., 2001).

One of the main driving forces for difference in community structure and composition are related to variations in nutrient availability (Leveau, 2006). These nutrient variations are related to differences in the cuticle properties, photosynthesis rate, leaf age and plant nutrition (Leveau, 2006). An important contributing factor to the viability of microbial communities in the phyllosphere is the opportunity and extent of nutrient leaching. Leveau (2006) stated that rain greatly increases the microorganism population likely because of increased nutrient availability as a result of induced leaching. It has also been observed that bacterial quantity and metabolic activity are significantly greater on wet leaves as opposed to dry leaves (Kinkel et al., 2000; Monier and Lindow, 2004). However, rainfall may also remove the microbial population if intense enough (Hirano; Leveau, 2006).

The cuticle layer, in particular, is an important determinant of microbial growth as it regulates nutrient and water availability (Leveau, 2006). Its main function is to protect the leaf

from the surrounding environment (Leveau, 2006). It is composed of cutin and waxes making it, to some degree, lipophilic and impermeable (Hirano and Upper, 2000). The cuticle limits diffusion of nutrients and water vapour and determines the hyrdophobicity of the leaf (Lindow and Brandl, 2003). The cuticle structure will vary with plant species and leaf age, and can be altered by epiphytes (Leveau, 2006). The cuticle can deter bacterial colonization by limiting nutrient diffusion and by inhibiting the wetting of the phyllosphere (Lindow and Brandl, 2003). However, not substantiated, it has been suggested that the cuticle, being made of carbon containing compound, could be a source of nutrients (Beattie, 2002).

Although, much effort has been placed into understanding the phyllosphere and its inhabitants many questions still remain. The relationship between the phyllosphere and the epiphytic community is not yet completely understood (Leveau, 2006). It has been found that some bacteria are able to inhabit the phyllosphere better than others (Arias, 1999). Many have reported similar bacterial species present on a wide variety of plants, suggesting that some species are well adapted to the phyllosphere environment. Some epiphytic bacteria have been found to provide important ecological functions, like fixing nitrogen (Sengupta et al., 1981), repelling insects (Battu and Arora, 1997) and as biocontrol agent (Leben, 1985). Studies have also found that bacteria are able to modify the phyllosphere environment (Beattie and Lindow, 2003).

Little is known about the diversity of microorganisms that inhabit the phyllosphere (Lambais et al, 2006). Microbial populations associated with the phyllosphere are often similar among plant species (Yang et al., 2001; Lambias et al., 2006). This suggests that specific plant traits select for particular microbial communities (Lambias et al., 2006; Yang et al., 2001), as well as influence the carrying capacity (Lindow and Bandl, 2003). Leveau (2006) suggested that if selection is possible it is likely based on leaf characteristics, like occurrence of protective sites, or environmental factors.

Microbial communities are good for measuring change as they quickly respond to temporal and spatial transformations (Garland, 1997). Different microbial community structure may affect ecosystem processes, like nutrients cycling and decomposition (Garland, 1997).

Microbial activity is a sensitive indicator of microbial response to changing environmental conditions (Boulton and Boon, 1991) and the rate of decomposition (Skambracks and Zimmer, 1998).

5.2 Objectives

The initial stage of decomposition usually results in a relatively fast loss in mass, dissolved organic matter (DOC and DON) and inorganic solutes (Berg 1984; Tietema and Wessel, 1994; Gosz et al., 1973; Yavitt and Fahey 1984). This is generally attributed to processes of leaching and microbial activity in terrestrial ecosystems. Although many studies have recognized the importance of leaching and microbial activity during this initial phase, a limited number of studies have addressed them in tandem (Yavitt and Fahey, 1986; Joergensen and Mayer, 1990; Tietema and Wessel, 1994). While preceding chapters have investigated the loss of leaf material through leaching, this chapter investigates the relationship leaching and microbial activity.

This study evaluates the response of phyllosphere microbes to litter trap conditions. The objective of this study was to determine if phyllosphere (leaf surface) microbes contribute to the decomposition of leaf litter collecting in litter traps. Two approaches were used to evaluate the contribution of microorganisms to the decomposition of freshly abscised sugar maple, basswood and beech leaf litter: (i) Microbial community structure was analyzed through community level physiological profiling at the end of the study period. The objective of this was to determine if microbial community structure was different between treatment and species. (ii) Microbial activity was measured as the evolution of CO_2 throughout the study period. The objective of this was to determine if microbes were active and how they responded to varying precipitation and temperature treatments (same treatments studied in chapters three and four). It was hypothesized that microbial community structure and activity would be greatest in basswood and sugar maple samples, and less in beech samples.

5.3 Material and Methods

5.3.1 Experimental Set-up

Freshly fallen and undamaged leaf-litter was collected between October 29th and October 30th 2007 from sugar maple, basswood and beech. The method of leaf litter collection was discussed previously in General Material and Methods (chapter one). Samples of 15 g (fresh weight) of leaf litter were first suspended over a plastic tub with netting. Four treatment levels were tested in triplicates (n=3) – low (L), medium (M), high (H) and freeze-thaw (F). The treatment applications were equivalent to a monthly precipitation amount of 30 mm, 60 mm and 100 mm. The treatment application was determined by dividing a monthly precipitation amount, low (30 mm), medium (60 mm) and high (100) mm, by 31 days (Figure 3.2). This was done to find the daily amount of precipitation that would be received assuming an equal amount of precipitation had fallen each day. Freeze-thaw treated leaf litter received the same amount of precipitation as the M treatment (60 mm) and was exposed to 0 °C on November 5th, 11th and 17th. After applying the treatments the litter was weighed and then placed into a 1 L jar. The jars were retrofitted and included a septum in the lid for gas extraction. All jars were kept in the dark and at 21 °C. The litter was weighed every other day (days when the lid was not sealed) and if the weight decreased it was amended by applying rainwater until the initial weight was reached again. Leaf litter incubation continued for a 26 day study period. Applying rainwater treatment to leaf litter stimulates microbial activity, and as such care was taken to leave time for the microbial activity to equilibrate before closing the jars for activity measurements.

5.3.2 Commuity Level Physiological Profiles (CLPP)

Following the 26 day incubation period community level physiological profiles (CLPP) were assessed using Biolog Ecoplate[TM] (Biolog Inc., CA, USA). The community level physiological profiles were only analyzed after the incubation experiment was completed. This followed the previously stated objective to determine if microbial communities were influenced by environmental conditions experienced by leaf litter while in litter traps. Samples from the same species and treatment were mixed together to form one sample. This was done because a single Biolog Ecoplate[TM] provides triplicates (n=3). A representative sample of 2 grams was

taken from this and cut into 2x2 cm squares and placed into a centrifuge tube. To the centrifuge tube, 10 ml of 0.85% NaCl solution was added and then subsequently shaken vigorously for 10 minutes to dislodge bacteria from the phyllosphere. A serial dilution was performed to reach a 1:10 000 concentration. From this 150 µl were added into each of the wells of the Ecoplate. The Ecoplate were incubated at 25 °C and the colour development of each well was read as optical density (OD) at 590 nm with a plate reader at time 0 and every 12 hours thereafter, up to 168 hours.

The microbial communities were characterized through overall rate of colour development (1); richness (R) (2); evenness of response (Shannon Index) (3); and the relative rate of substrate utilization (4) (Garland, 1997):

$$(1) \ Rate \ of \ Colour \ Development = \frac{optical \ density \ (OD) at \ 590nm}{time \ (hr)}$$

$$(2) \ Microbial \ Richness \ (R) = number \ of \ oxidized \ Carbon \ substrates \ (> 0.25OD)$$

$$(3) \ Shannon \ Index \ (H) = -\sum Pi \ (\ln Pi)$$

$$(4) \ Average \ Well \ Colour \ Development \ (AWCD) = \sum \frac{ODi}{31}$$

where Pi is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates ($\sum ODi$).

The threshold for a positive test was defined as the positive value after background correction of 0.25 absorbency (abs.) (Garland, 1997). Optical density data was corrected by blanking each response well against its first reading immediately following inoculation (Insam and Goberna, 2004). Blanking against the first reading (t=0) eliminates absorbance of the carbon reading and negative values often associated with subtracting the control well from the response well (Insam and Goberna, 2004). The well response was not standardized as it was important to take into consideration the response of the community per unit habitat (Garland, 1997).

5.3.3 Microbial Respiration

Once the litter was placed in the incubation jars, the jars were sealed and placed in the dark at 21 °C, after 24 hours. A 250 µl sample was taken from the sealed jar through the septum

and analyzed using an HP6890 gas chromatographer for Carbon Dioxide (CO_2) and Nitrous Oxide (N_2O) evolution at time 0 and 24 hours. Throughout the 26 day incubation period the sampling sequence was: from 0 to 24 hours with the lid loosely sealed, 15 minutes the lid was open and from 24 (minus the 15 minutes) to hour 48 the lid was tightly sealed. This sampling sequence continued throughout the study period. The gas was sampled immediately after tightly closing the lid (hour 24 of the sampling procedure) for the initial concentration and then after 24 hours of the lid being closed (hour 48 of the sampling procedure) to determine the amount evolving within the 24 hour period. The daily CO_2 flux was determined using the following equation adapted from Hogg et al. (1992):

$$(5) \quad R(\mu g\ CO2/g/d = (Cs - Ca)VD/(M/t)$$

where R ($\mu g\ CO_2\ g^{-1}\ d^{-1}$) is the amount of CO_2 (μg) evolved per gram of dry material (in this case leaf litter); Cs is the concentration of CO_2 from the leaf litter ($\mu L/L$); Ca is the concentration of CO_2 ($\mu L/L$) from the control sample; V is the volume of effective headspace (L); D is the density of CO_2 adjusted for temperature, pressure and humidity (g/L); M is the dry mass of the sample (G); and t is the sampling time in interval in days.

Henceforth the samples will be abbreviated to include both the species and treatment type. Sugar maple, basswood and beech samples will by denoted by M, B or Be, respectively. The treatments low, medium, high and freeze-thaw will be denoted as L, M, H and F, respectively. For example, if the sample in context is sugar maple exposed to medium treatment it will be denoted as MM.

5.4 Statistical Analysis

A one-way ANOVA was completed to compare the change in C and N between treatments, as well as in comparison to the baseline (freshly abscised leaf litter collected on October 22nd and 24th 2007). All data sets were examined for homogeneity of variances between the two sets of compared data (using the Levene Test) and normal distribution was tested using Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test (Dytham, 2003). The variance of the data is homogeneous, normally distributed and the data is continuous. For all statistical

analyses the threshold probability level for determining significant differences was $p < 0.05$ and $p < 0.10$.

To determine if there is a difference between treatment leachate concentrations over the study period repeated measures of ANOVA was completed. Repeated measures of ANOVA was completed to compare each of the measured evolution of CO_2 between treatments over the study period. Sampling time (over the 26 day study period) was the repeated (within subjects) factor and CO_2 evlotuion was the main (between subjects) factor. All data sets were examined for homogeneity of variances between the two sets of compared data (using the Levene Test) and normal distribution was tested using Kolmogorov-Smirnov (K-S) Test and the Shapiro-Wilks Test (Dytham, 2003). The variance of the data is homogeneous, normally distributed and the data is continuous. Significance levels were adjusted using the Greenhouse-Geisser and Huyuh-Feldt to account for violations in sphericity. With limited statistically significant results it was deemed prudent to advance this research through the use of descriptive statistics. This decision is supported by Dytham (2003) who argued that the use of descriptive statistics is important for they often reveal stories and trends that often go unnoticed.

5.5 Results and Discussion

5.5.1 Community Level Physiological Profiling

The rate of colour development was measured from the initial point of inoculation, every 12 hours, up to 132 hours (Figure 5.1). The rate of colour development is based on the average well colour development (AWCD) (Garland, 1997). Differences in rate of colour development are dependent on inoculums density (Garland, 1997). In this study, the rate of colour development was related to litter type (species) and treatment application. Sugar maple litter exhibited the lowest rate of colour development, in comparison to beech and basswood. Basswood and beech samples showed similar rates of colour development. Sugar maple samples displayed the greatest responses in L, followed by H and M (which were very similar), and then F (L>H>M>F). Basswood samples displayed the greatest response in F samples, followed by L, M and then H (F>L>M>H). The beech samples' results differed substantially. Beech samples treated with M had the greatest rate of colour development, followed by L and H and then F

(M>L>H>F). Since the rate of colour development is directly proportional to the inoculums density (Garland, 1997), it is fair to assume that basswood and beech samples harbored a more favourable environment for microbial growth over sugar maple litter. This is contradictory to previous chapter results. Specifically, basswood and sugar maple exhibited a decrease in weight, suggesting a greater extent of decomposition, while beech did not exhibit a decrease in weight suggesting that little decomposition had transpired. The results of the microbial response to species suggest that maple likely lost the majority of weight through leaching as opposed to microbial activity.
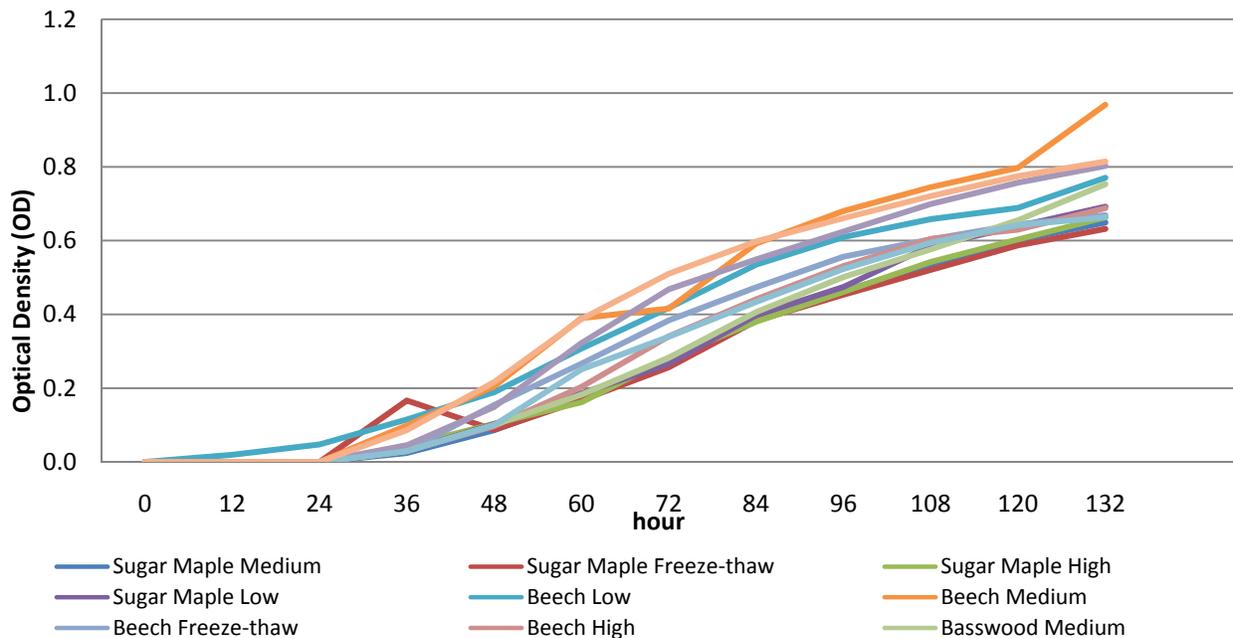
Figure 5.1: Rate of colour development over a 132 hour inoculation period. The first part of the name in the legend refers to the species and the second part refers to the treatment.

Similarly, AWCD was greatest in basswood, and subsequently lower in beech and then sugar maple (Figure 5.2). Within treatments, AWCD did not display a particular trend. Sugar maple samples displayed the following trend, L>H>M>F, while basswood sample exhibited L>F>H>M. Beech samples responded much differently, namely M>L>F>H. With the exception

of basswood, sugar maple and beech exhibited the same trends in AWCD as in rate of colour development. However, these results do not coincide with microbial respiration results, with the exception of one case. Basswood displayed an increase in microbial activity in freeze-thaw treatments and displayed a greater AWCD in F samples over treatment M. As well, sugar maple displayed an increase in microbial activity in the medium samples over freeze-thaw treatment and similarly displayed an increase in AWCD in M over F.

Similar to average well colour development, microbial richness (R) and the Shannon Index (H)[1] were greatest in basswood samples, followed by beech and sugar maple. In all of the treatments, AWCD, R and H showed a proportionate relationship, such that treatments that displayed a high AWCD also displayed a high R and H (Figure 5.2).
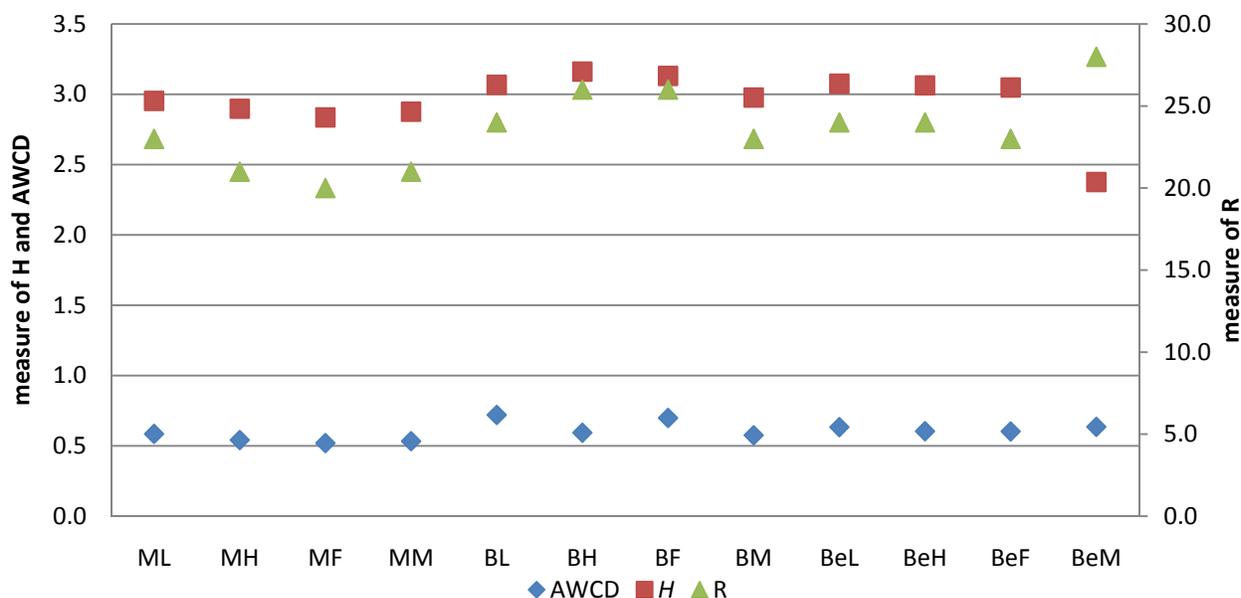
Figure 5.2: Average well colour development (AWCD), richness (R) and Shannon Index (H) at t=108 hours for each of the measured treatments (L, M, H and F) for sugar maple (M), basswood (B) and beech (Be) leaf litter.

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[1] It is standard for the Shannon Index to be denoted by 'H', however to this point in this study 'H' has stood for high, with regard to the level of precipitation. Therefore, an italicized 'H' will denote the Shannon Index for the remainder of this study.

Principal components analysis (PCA) was performed to determine if C source metabolism differed between species and treatment (Figure 5.3). Data was normalized to correct for inoculums density differences with AWCD (Garland, 1997). When comparing all of the species and treatments together, the first principal component (PC) explains 45% of the variance, while the second and third PC's explain 18% and 11%, respectively. Samples BF, BL, BeF and BeL formed one distinct cluster, and samples BM, MM, MH, ML and MF formed another distinctive cluster. Samples BeH, BH and BeM were situated in a relatively dispersed pattern, distant from all other samples.

Observed carbon source utilization profiles indicated a distinct microbial metabolic response related to species and moisture level. However, inconsistency in microbial response to moisture level is contradictory to previous research.
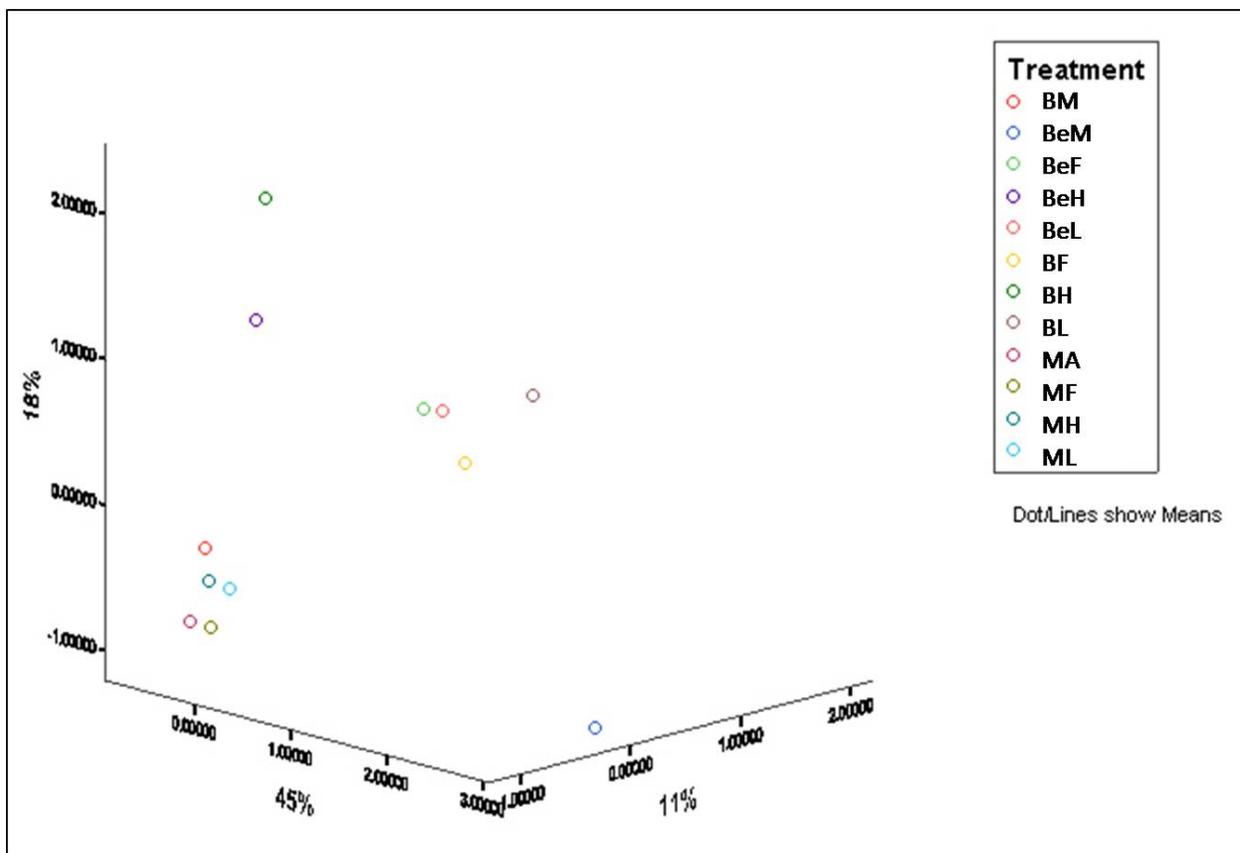
Figure 5.3: Principal component analysis on carbon source utilization of Biolog Ecoplate for each of the studied treatments (L, M, H and F) for sugar maple, basswood and beech leaf litter.

5.5.2 Microbial Respiration

In general, microbial respiration was greatest during the initial phase of the experiment, day one to day six. After this initial period, the respiration substantially fell over the following 6 days. From day 12 to day 20 the respiration increased minimally and began to plateau. All three litter species were within a similar range, between approximately 5 and 35 $\mu g\ CO_2\ g^{-1}d^{-1}$. Overall sugar maple displayed the highest levels of respiration, followed by beech and then basswood, although beech and basswood were very similar.

Microbial activity did not correspond with treatment type as no distinguishable or consistent trend could be found within or between species. Microbial respiration levels of basswood and beech CO_2 evolution greatly fluctuated over the study period. Basswood leaf litter exposed to treatment H differed significantly ($p<0.05$) from basswood samples treated with L and M. Sugar maple respiration levels were very similar between treatments. Thus, sugar maple phyllosphere microbes were not influenced by varying precipitation and temperatures levels. Microbial respiration rates correspond to the loss of weight measured in earlier chapters. Microbial respiration was highest in sugar maple and basswood, and lowest in beech.
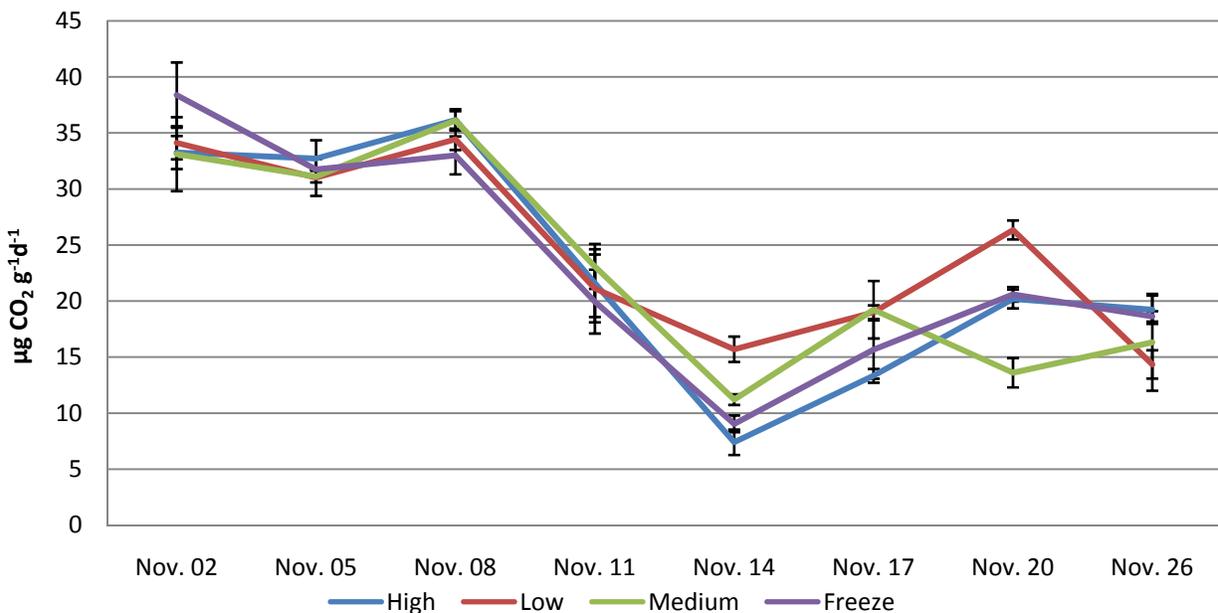
Figure 5.4: Microbial activity measured as evolution of CO_2 over a 26 day study period from sugar maple leaf litter treated with H, L, M and F.
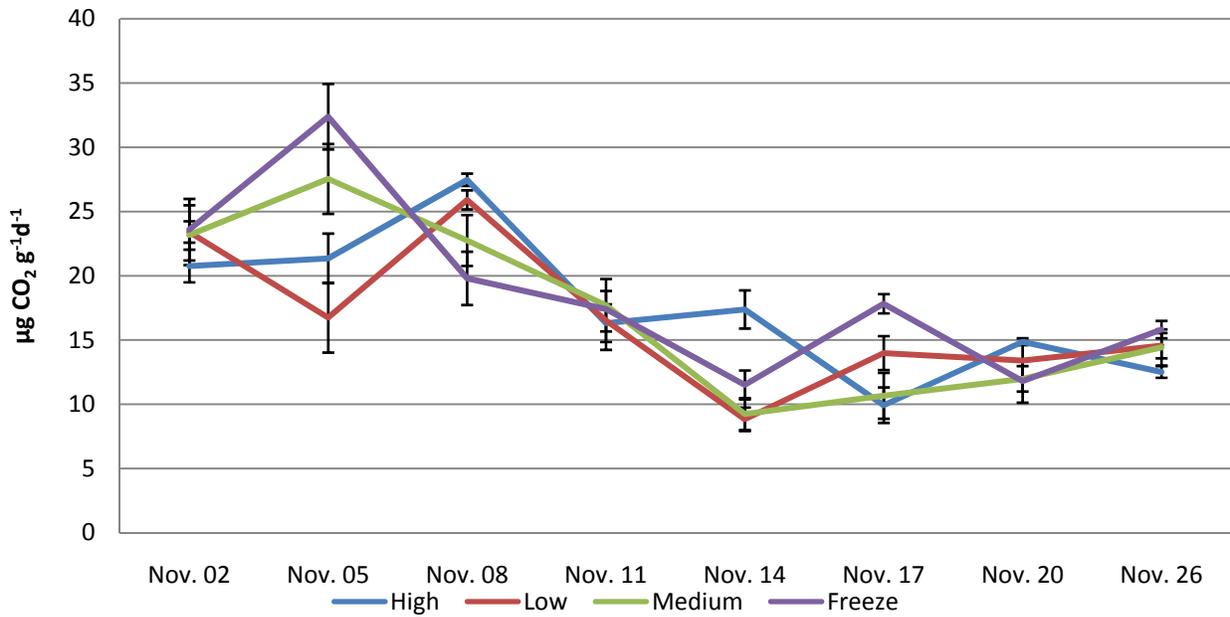
Figure 5.5: Microbial activity measured as evolution of CO_2 over a 26 day study period from basswood leaf litter treated with H, L, M and F.
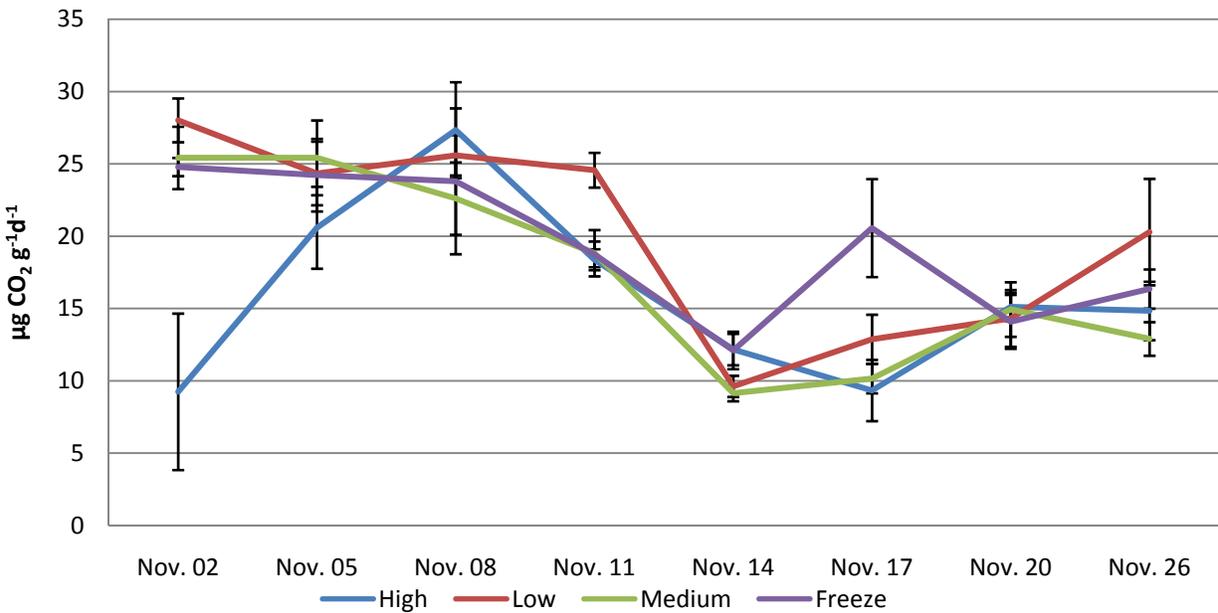
Figure 5.6: Microbial activity measured as evolution of CO_2 over a 26 day study period from beech leaf litter treated with H, L, M and FT.

5.5.3 Nutrient Analysis

Initial C and N results determined basswood leaf litter to have the lowest C/N ratio, followed by sugar maple and beech. Treated litter displayed a change in C/N ratios; however basswood continued to have the lowest C/N results followed by sugar maple and then beech (Table 5.1). Treated basswood and sugar maple leaf litter had a decrease in the C/N ratio mostly due to an increase in N, similar to chapter three. Basswood samples treated with M had the largest decrease followed by F, L and then H. Sugar maple samples had the lowest C/N ratio in freeze-thaw treated litter followed by H, L and then M (F>H>L>M). Beech leaf litter exhibited a substantial decrease in the C/N ratio of freeze-thaw treated samples but an increase in the H, M and L (H>M>L). Beech freeze-thaw treated samples exhibited an increase in N, while H, M and L exhibited a decrease in N.

Table 5.1: Carbon and Nitrogen (mg/kg) results from post- incubation experiment for sugar maple, basswood and beech.

Species	Treatment	C (mg/kg)	N (mg/kg)	C/N
Sugar Maple	H	44.22	0.85	52.02
	M	44.67	0.78	57.27
	L	45.01	0.85	52.95
	F	45.89	1.00	45.89
	C	44.52	0.63	70.67
Basswood	H	44.87	1.31	34.25
	M	45.12	1.65	27.35
	L	45.02	1.52	29.62
	F	44.71	1.59	28.12
	C	44.73	1.58	28.31
Beech	H	48.67	0.70	69.53
	M	47.99	0.76	63.14
	L	48.49	0.85	57.05
	F	48.61	1.04	46.74
	C	48.06	0.87	55.24

Litter quality, microbial activity and microbial structure did not correspond. It was hypothesized that a low C/N ratio would harbor more microbial activity. Overall, basswood had the lowest C/N ratio, the greatest community dynamics and high microbial activity (higher then beech and same as sugar maple), followed by sugar maple and then beech.

None of the measured variables (microbial activity, community structure or C/N ratio) directly related to the moisture level. No trend could be determined based on microbial activity, community structure and changes to the C/N ratio and the applied treatment.

The findings of this study contradict many studies that have reported an increase in microbial activity in response to increased moisture levels (Taylor and Parkinson, 1988c; Howard and Howard, 1993; Vanhala, 2002). The lack of response to increased moisture level in this study suggests that under these conditions moisture level did not dictate litter decay. In general, litter decomposition is dependent on moisture, temperature, and physical and chemical characteristics of the litter (Edmonds 1980). Taylor and Parkinson (1988c) found that large differences in moisture level were required to produce different decomposition rates, and thus temperature was a more important determinant of decomposition over moisture levels. Schlentnen and Van Cleve (1985) stated that at high moisture levels microbial activity was more responsive to temperature changes. The unexpected results of this study may either be due to similar moisture regimes or high moisture regimes. However, Schlentner and Van Cleve (1985) stated that at 10-20˚C microbial activity is more responsive to moisture changes. This may suggest that the quality of the litter was the determining characteristic. Swift et al. (1979) found that litter quality was more important than climatic conditions in determining the decay rate. Swift et al. (1979) reported that the decomposition of litter from different species was dissimilar regardless of the same climatic conditions. The rate of decomposition is inversely related to the lignin and C:N ratio and is directly related to the N and P concentrations (Flanagan and Van Cleve, 1983). Therefore microbial activity is greatly dependent on the quality of litter (Flanagan and Van Cleve, 1983). Microbial activity was related to the initial C/N ratios. Microbial activity was higher in sugar maple and basswood samples then beech samples, as was the initial C/N ratio. In earlier chapters basswood was the only species to have measurable levels of P. Although

109

P was not measured in this experiment it is possible that it was higher which would explain why basswood leaf litter exhibited a greater microbial dynamics.

In a study conducted by Aerts and de Caluwe (1997) it was found that the initial rate of litter respiration was nutrient controlled. Specifically, the initial rate of respiration is dependent on the easily decomposable compounds, and thus dependent on the amount of labile substances in the litter of each of the species (Aerts and de Caluwe, 1997). Ryan (2005) also stated that substrate strongly controlled respiration, but is also related to soil moisture.

This study's trend of respiration rate observed over the 26 day period is inconsistent with the results reported by Taylor and Parkinson (1988c). Taylor and Parkinson (1988c) reported that aspen litter exposed to medium (30 ml/week) and high (60 ml/week) watering rates stimulated increasing respiration rates when incubated at 18˚C and 26˚C for at least the first 20 days of the study. In this study the temperature was kept at 21˚C. Thus it is reasonable to believe that similar results should have been observed. However, this was not the case and this study's findings resembled the finding of Taylor and Parkinson's (1988c) 2˚C and 10˚C treatments. Under this treatment the respiration rate was observed to decrease for at least the first 20 days in low (15ml/week), medium and high watering conditions (Taylor and Parkinson, 1988c). This was presumed to be due to saturated condition (Taylor and Parkison, 1988c). Similar to this study, litter leveled off after the initial decrease (Taylor and Parkinson, 1988c). Therefore, it may be that this study over watered the leaf litter throughout the incubation period also producing saturated conditions.

Sugar maple samples exposed to freeze-thaw conditions exhibited a significant ($p<0.10$) decrease in respiration in comparison to the M, suggesting that microbial activity was hindered. Kowal (1969) found leaching to significantly relate to mass loss and CO_2 production. In the proceeding chapter, sugar maple litter showed an increase in inorganic nutrient leaching (K, Ca and Mg) and weight loss due to exposure to freeze-thaw cycles, but did not exhibit an increase in DOC or DON leaching. This may suggest that although the leaf litter was structurally affected by freeze-thaw treatment, microbes associated with sugar maple leaf litter were not. As well, Schimel and Clein (1996) deduced that a decrease in respiration below control is likely due to a reduced ability of microbes to metabolize C and respire.

Following a thaw, basswood and beech generally displayed an increased flux of microbial respiration, with the exception of day nine. However, only FT treated beech samples significantly ($p<0.05$) differed from M treated beech samples. Many studies have reported an initial flux of CO_2 following a thaw (Vanhala; Schimel and Clein, 1996; Neilsen et al., 2001; Teepe and Ludwig, 2004). Specifically, a similar trend was reported by Schimel and Clein (1996). As previously stated, Schimel and Clein (1996) suggest that the flux is due to metabolism of organic substances released from microorganisms that died as a result of the freeze-thaw treatment. As well, each subsequent freeze-thaw treatment produced less of a response then the previous application. Schimel and Clein (1996) described a similar occurrence, relating to less biomass being killed with each successive cycle. Neilson et al. (2001) also found an increase in microbial respiration when freezing was induced. However, Neilson et al. (2001) did not measure a decrease in microbial biomass, thus it was concluded that induced freezing cause a release C from the soil.

Although many studies have reported increased nitrous oxide (N_2O) emission following freeze-thaw conditions (Neilsen et al., 2001; Ryan, 2005; Matzner and Borken, 2008), this study did not observe such an event, per se. Similar to CO_2 emissions, sugar maple litter produced less N_2O when exposed to freeze-thaw treatment, as compared to medium treatment. Basswood and beech leaf litter displayed increased N_2O emissions initially but did not exhibit increased peaks with later freeze-thaw treatment. Teepe and Ludwig (2004) found that fluxes of N_2O from forests soils differed considerably depending on site conditions, such as dominant plant species. Teepe and Ludwig (2004) observed an increase in beech dominated areas, but not in aspen. Many of the studies reporting an increase in N_2O are conducted on soil. Matzner and Borken (2008) stated that the increase in C and N from thawing soil is often related to microbial biomass, death of roots and changes in the soil structure. As stated, microbial biomass and structural changes can explain results from freeze-thaw activity on leaf litter. However it is likely that the results from soil systems provide greater response to freeze-thaw, at least with respect to structural changes due to the complexity of soil structure. For example, Matzner and Borken (2008) reported that some studies associated the increased flux of N_2O from sites where production of gases built up

111

during frost. Furthermore, many studies have reported increased N mineralization associated with freeze-thaw treatment (Matzner and Borken, 2008 and Neilson et al., 2001).

5.6 Summary and Conclusions

It was the purpose of this chapter to determine if microbes contribute to changes in the sugar maple, basswood and beech leaf litter collecting in litter traps. The results of this experiment indicate that microbes are present and are active in the leaf litter. Increases in the N content of leaf litter imply that microbes may have an effect on the leaf litter. Microbial activity and community structure did not increase with increased precipitation levels. Over the study period microbial activity decreased. This is likely due to a lack of readily decomposable material available. In the initial stages of the study microbes are highly active. As the amount of leachable material decreases so does the microbial activity until reaching a steady state.

The results of this study find that the initial concentration of C and N (litter quality) are better predictors of microbial activity then environmental conditions. The difference observed in the three species is likely related to the microbial community structure. Many studies have found contrasting results related to the manner in which microbes respond to similar environmental conditions. As well, this study's findings indicate that microbial activity will affect leaf litter collecting in litter traps. Specifically, sugar maple, basswood and beech leaf litter exposed to 0˚C and 21˚C and 30mm, 60mm and 100 mm moisture levels.

Chapter 6
Final Summary and Conclusion

6.1 Summary and Conclusion

The purpose of this research was to determine if litter fall collecting in litter traps undergoes initial stages of decomposition. As such, this study asked the research question: Does sugar maple (*Acer saccharum* Marsh.), american basswood (*Tilia Americana* L.) and american beech (*Fagus grandifolia* Ehrh.) leaf litter collected from litter traps after a two week period undergo change in comparison to freshly abscised leaf litter?

Two pivotal studies contributed to the development of this research question. Ukonmaanaho and Starr (2001) measured the in-situ flux of nutrients from leaf litter collecting in litter-traps with the intent to determine if leaf litter nutrient contributions were underestimated. Ukonmaanaho and Starr (2001) positively identified that nutrient cycling studies underestimated the return of nutrients from leaf litter. Similarly, Oelbermann (1999) conducted an ex-situ study to determine if leaf litter collecting in litter traps, when exposed to precipitation, would undergo weight and nutrient loss from leaching. However, Oelbermann (1999) found that, for the most part, leaf litter studies likely overestimated the amount of nutrients entering the system. Further research was required to find a consensus.

A preliminary study (Chapter 2) was conducted to measure whether under in-situ conditions sugar maple, basswood and beech leaf litter in litter traps, for a 14 day period, underwent change in comparison to freshly abscised leaf litter. For the most part, the results determined that the leaf litter nutrient content and dry weight would be underestimated. Specifically, sugar maple litter collected from the traps led to an underestimation of magnesium(Mg) by 31.1%, Calcium (Ca) by 25.1% and dry weight by 12.4%. Potassium (K) and phosphorus (P) were no longer detected. Assuming the concentrations were just below detection, K was underestimated by 33.9% and P by 14.0%. Nitrogen (N) increased by 4.47% and carbon (C) remained constant. Basswood litter collected from the traps led to an underestimation of Mg by 4.9%, Ca by 9.8%, K by 23.8%, P by 3.7% and dry weight by 4.65%.

Nitrogen increased by 1.10% and C remained constant. Beech litter collected from the traps led to an underestimation Mg by 10.5%, Ca by 12.3%, K by 6.5% and dry weight by 0.16%. Phosphorus was not detected in freshly abscised leaves or leaves from the litter trap. N decreased by 16.5% and C remained the same. Leaf litter from different species responded differently while in litter traps when exposed to the same environmental conditions.

The results of this preliminary study indicated that leaf litter collecting in litter traps experienced initial stages of decomposition as a result to its exposure to precipitation, freezing and thawing temperatures, and microbial decomposition. This study also evaluated litter decomposition using an an ex-situ approach to evaluate the relationship between precipitation, freezing and thawing temperatures, microbial activity and the measured decomposition of litter.

The first of these studies measured the change in dry mass and nutrient content of sugar maple, basswood and beech leaf litter exposed to varying levels of precipitation. The intention was to evaluate the response of leaf litter to precipitation, and determine if leaf litter from different deciduous species respond differently. The results of this study (Chapter 3) determined that precipitation influenced sugar maple, basswood and beech leaf litter in litter traps left only for a two week period as it induced a decrease in dry weight. However, the influence of precipitation on the nutrient content was variable. The degree to which the element increased or decreased did not relate to the precipitation magnitude. The lack of an identifiable trend allows for only general conclusions. The results state that K and Mg were readily leached from all species. Phosphorus was undetectable after the 14-day study period in sugar maple and beech but increased in basswood. The Ca content of the leaf litter increased. It is assumed that Ca would continue to increase, via immobilization, until the structural components of the leaf litter began to decompose (e.g. Attiwill, 1967).

The initial C/N ratio, leaf physical characteristics and the level of degradation of the litter can help to determine the influence of precipitation on leaf litter. Sugar maple exhibited the greatest decrease in weight due to leaching of organic matter (DOC and DON). The considerable influence of precipitation on sugar maple is likely because it does not have a waxy or tough cuticle and showed signs of physical deterioration. Basswood was also greatly affected by

114

precipitation. This may be due to its relatively low C/N ratio. Beech leaf litter was moderately affected. This may be due to its thick and waxy cuticle, and high C/N ratio.

Starr et al. (2007) stated that precipitation reaching the forest floor is significantly different from precipitation collected from an open area. In this study, rainfall was collected from an open area. In a forest ecosystem, or in-situ, it is likely that leaf litter in litter traps would be intercepted by throughfall and stemflow. Further analysis should be conducted to investigate the importance of throughfall in manipulating the nutrient content of leaf litter. It is hypothesized that the litter would be further enriched through deposition from throughfall and stemflow. Effort should be taken to collect rainwater from the location the litter was taken. Not only to simulate similar nutrient fluxes from throughfall, but also to minimize variability in atmospheric deposition.

The second of these studies measured the change in dry mass and nutrient content of sugar maple, basswood and beech leaf litter exposed to freeze-thaw temperatures. The intention was to evaluate the response of leaf litter to freeze-thaw and determine if leaf litter from different deciduous species respond differently. Results of this study (Chapter 4) determined that freeze-thaw influences sugar maple, basswood and beech leaf litter in litter traps. Freezing and thawing leaf litter induced a greater decrease in weight in comparison to the control samples (litter not exposed to freeze-thaw conditions), with the exception of beech. The influence of freezing and thawing leaf litter was proportional to the initial C/N ratio. Unlike the literature, this study did not find a significant increase in C and N decomposition and nitrous oxide (N_2O) production. The literature did state that cycles of freeze-thaw accelerate litter decomposition (Campbell et al., 2005; Matzner and Borken, 2008) through structural disruption and membrane rupture (Tukey 1966; Burke et al., 1976; Hurst et al., 1985). This study found a decrease in N and Ca in sugar maple and basswood leaf litter. This is markedly different from chapter three and is likely due to structural damage induced by freeze-thaw. Beech exhibited an increase in N and Ca suggesting that it did not undergo structural damage.

As stated earlier, it is difficulty in predict the affect of freeze-thaw on leaf litter due to the number of variables that must be taken into account. Taylor and Parkinson (1988e) noted a

number of factors to consider including species, moisture content, temperature range (specifically the final low temperature), rate of temperature change, duration of freezing and thawing periods and the number of repeated cycles. This study did not take the rate of temperature change and moisture content into consideration. In future studies effort should be made to mimic these parameters more accurately.

The last of these studies measured the response of phyllosphere microbes to litter-trap conditions. The intent of the studies was to determine if microbes contribute to changes in leaf litter collecting in litter traps. As well, this study proposed to evaluate the relationship between phyllosphere microbial activity, varying precipitation levels and freeze-thaw temperatures. The results of this study indicated that microbes are present and are active in the leaf litter. Microbial activity and community structure did not increase with increased precipitation levels. Over the study period microbial activity decreased. Similar to earlier studies, this study also found that the initial concentration of C, N and P are better predictors of microbial activity than environmental conditions.

Although many studies reported increased N_2O emission following freeze-thaw conditions (Neilsen et al., 2001; Ryan, 2005; Matzner and Borken, 2008), this study did not. Similar to CO_2 emissions, sugar maple litter produced less N_2O when exposed to freeze-thaw treatment, as compared to the control. Basswood and beech leaf litter displayed increased CO_2 and N_2O emissions initially but did not exhibit increased peaks with later freeze-thaw treatment.

The greater microbial community structure and microbial response to freezing conditions of beech leaf litter over sugar maple litter does not correspond with other findings of this study. The characteristics of beech leaf litter suggest that it is a less favorable environment for microbial growth. Further research is required to determine the relationship between microbial community structure and leaf litter characteristics. As well, this study assumed that the microbial community structure changed over the study as samples exposed to different treatments reported different dynamics. In order to better understand the behavior of phyllosphere microbial communities further research is required to map the change in community structure.

116

6.2 Implication of this Research

The overall objective of this study was to devise a correction that would take into account the changes in leaf litter collecting in litter traps so to provide an accurate representation of freshly abscised leaf litter. Meeting this proposed objective proved to be difficult. The variability in species response to treatments and in the measured constituents (C, N, P, K, Ca and Mg) obscured an opportunity to determine a significant trend.

The results of this study indicated that leaf litter collecting in litter traps was influenced by precipitation, freezing and thawing temperatures and microbial activity. Many of the results of this study were similar to litter bag decomposition studies (e.g. Gosz et al., 1973, Parsons et al., 1990, Huang and Schoenau, 1997). The results of this study indicate that changes in leaf litter collecting in litter traps observed over a two week period is equivalent to the decomposition of leaf litter in the initial stages of decomposition. This suggests that initial stages of decomposition are not dependent on ground level processes (e.g. soil microbial activity). Most of the change is a result of leaching and the microbial demand. Thus, the change in the litter is dictated by litter quality and leaf structural properties (e.g. thickness of cuticle) more than the climatic conditions tested in this study. The results of this study suggested that leaf litter similar to sugar maple and basswood may experience considerable change while in litter traps, even over a 14-day period. Leaf litter similar to beech can tolerate longer periods in the litter trap without suffering significant change, at least over a 14-day period.

6.3 Recommendation for Future Research

This study measured the influence of leaving leaf litter in litter trap for 14-days. Many studies have reported leaving the litter in litter traps for longer. Further research into a temporal pattern of litter decomposition while in litter traps may help to define an appropriate length of time for litter to be in litter traps.

Many studies reported that mixing different species of litter increases the decomposition of leaf litter. Since a number of litter trap studies are interested in collecting a representative sample of the canopy, effort is not taken to reduce species mixing. Further research is required to

117

understand and predict the influence of mixing different species on the decomposition of litter while in litter traps.

Academic literature is significantly lacking attention on topics of freeze-thaw and phyllosphere activity on the decomposition of leaf litter. The majority of the present literature looks at the affects of freeze-thaw and microbial activity on the soil profile. As a result, this study formed an exploratory approach to these ideas. Further research is required to understand the influence of freezing and thawing temperatures and phyllosphere activity strictly on leaf litter.

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Appendix

The following is the specifications for the Gas Chromatography HP6890 which was used to analyze for CO_2 and N_2O in Chapter 5. This also outlines the method information for the method that I produced to analyze for CO_2 and N_2O.

Method: C:\CHEM32\1\METHODS\GHG.M
Modified: 6/10/2008 at 7:15:11 PM

Injection Source and Location

Injection Source: Manual

Injection Location: Back
==
=====
6890 GC METHOD
==
=====

OVEN
  Initial temp: 35 'C (On)   Maximum temp: 325 'C
  Initial time: 10.00 min   Equilibration time: 0.50 min
  Ramps:
   # Rate Final temp Final time
   1   0.0(Off)
  Post temp: 50 'C
  Post time: 0.00 min
  Run time: 10.00 min

FRONT INLET (PURGED PACKED)   BACK INLET (SPLIT/SPLITLESS)

Initial temp: 50 'C (Off)   Mode: Splitless

Flow: 3.1 mL/min (Off)   Initial temp: 200 'C (On)

Gas type: Helium   Pressure: 9.20 psi (On)

Purge flow: 25.0 mL/min

Purge time: 0.00 min

Total flow: 38.0 mL/min

Gas saver: On

Saver flow: 20.0 mL/min

Saver time: 2.00 min

Gas type: Helium

COLUMN 1   COLUMN 2

Capillary Column   Capillary Column

Model Number: Agilent 19091J-413   Model Number: J&W 19095P-Q04

HP-5 5% Phenyl Methyl Siloxane   HP-PLOT

Max temperature: 325 'C   Max temperature: 290 'C

Nominal length: 30.0 m   Nominal length: 30.0 m

Nominal diameter: 320.00 um   Nominal diameter: 530.00 um

Nominal film thickness: 0.25 um   Nominal film thickness: 40.00 um

Mode: constant pressure   Mode: (see column 1)

Pressure: 9.20 psi   Pressure: 9.20 psi

Nominal initial flow: 2.1 mL/min   Nominal initial flow: 8.1 mL/min

Average velocity: 33 cm/sec   Average velocity: 66 cm/sec

Inlet: Back Inlet   Inlet: Back Inlet

Outlet: Front Detector   Outlet: Back Detector

Outlet pressure: ambient   Outlet pressure: ambient

FRONT DETECTOR (FID)   BACK DETECTOR (TCD)

Temperature: 250 'C (On)   Temperature: 225 'C (On)

Hydrogen flow: 35.0 mL/min (On)   Reference flow: 16.0 mL/min (On)

149

Air flow: 200.0 mL/min (On)   Mode: Constant column+makeup flow

Mode: Constant makeup flow   Combined flow: 12.0 mL/min

Makeup flow: 0.2 mL/min (On)   Makeup flow: On

Makeup Gas Type: Helium   Makeup Gas Type: Helium

Flame: On   Filament: On

Electrometer: On   Negative polarity: Off

Lit offset: 0.5

SIGNAL 1   SIGNAL 2

  Data rate: 20 Hz   Data rate: 5 Hz

  Type: front detector   Type: back detector

  Save Data: On   Save Data: On

  Zero: 0.0 (Off)   Zero: 0.0 (Off)

  Range: 0   Range: 0

  Fast Peaks: Off   Fast Peaks: Off

  Attenuation: 0   Attenuation: 0

COLUMN COMP 1   COLUMN COMP 2

  Derive from front detector   Derive from back detector

POST RUN

Post Time: 0.00 min

TIME TABLE

  Time   Specifier   Parameter & Setpoint

GC Injector

  Front Injector:

No parameters specified

  Back Injector:

No parameters specified

==

                   Calibration Table

==

Calib. Data Modified :   5/7/2008 5:23:12 PM

Calculate   :   External Standard
Based on   :   Peak Height

Rel. Reference Window :   5.000 %
Abs. Reference Window :   0.000 min
Rel. Non-ref. Window :   5.000 %
Abs. Non-ref. Window :   0.000 min
Use Multiplier & Dilution Factor with ISTDs
Uncalibrated Peaks   :   not reported
Partial Calibration   :   Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type   :   Linear
Origin   :   Included
Weight   :   Equal

Recalibration Settings:
Average Response   :   Average all calibrations
Average Retention Time: Floating Average New 75%

Calibration Report Options :
  Printout of recalibrations within a sequence:
    Calibration Table after Recalibration
    Normal Report after Recalibration

If the sequence is done with bracketing:

Results of first cycle (ending previous bracket)

Signal 1: NEW,

Signal 2: FID1 A,

Signal 3: TCD2 B,

RetTime Lvl Amount   Height   Amt/Height Ref Grp Name
 [min] Sig   [ng/ul]
-------|--|--|----------|----------|----------|---|--|---------------
 1.258 3 1 100.00000 2937.59985 3.40414e-2   CO2
 1.394 2 1   5.00000   3.72815   1.34115   CH4
 1.883 3 1   10.00000   7.05710   1.41701   N2O
===
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