Leaf Litter Decomposition in Vernal Pools of a Central Ontario Mixedwood Forest

by

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LEAF LITTER DECOMPOSITION IN VERNAL POOLS OF A CENTRAL ONTARIO MIXEDWOOD FOREST

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University of Guelph, 2012

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Dr. Jonathan Schmidt
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Vernal pools are small, seasonally filling wetlands found throughout forests of north eastern North America. Vernal pools have been proposed as potential 'hot spots' of carbon cycling. A key component of the carbon cycle within vernal pools is the decomposition of leaf litter. I tested the hypothesis that leaf litter decomposition is more rapid within vernal pools than the adjacent upland. Leaf litter mass losses from litterbags incubated in situ within vernal pools and adjacent upland habitat were measured periodically over one year and then again after two years. The experiment was carried out at 24 separate vernal pools, over two replicate years. This is a novel degree of replication in studies of decomposition in temporary wetlands. Factors influencing decomposition, such as duration of flooding, water depth, pH, temperature, and dissolved oxygen were measured. Mass loss was greater within pools than adjacent upland after 6 months, equal after 12 months, and lower within pools than adjacent upland after 24 months. This evidence suggests that vernal pools of Central Ontario are 'hot spots' of decomposition up to 6 months, but not after 12 and 24 months. In the long term, vernal pools may reduce decomposition rates, compared to adjacent uplands.
ACKNOWLEDGEMENTS

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<tr>
<td>% day⁻¹</td>
<td>percent mass loss per day</td>
<td>49</td>
</tr>
<tr>
<td>AET</td>
<td>actual evapotranspiration</td>
<td>23</td>
</tr>
<tr>
<td>AFDM</td>
<td>ash-free dry mass</td>
<td>48</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
<td>49</td>
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<tr>
<td>C</td>
<td>carbon</td>
<td>15</td>
</tr>
<tr>
<td>C:N</td>
<td>carbon to nitrogen ratio within litter</td>
<td>18</td>
</tr>
<tr>
<td>Ca</td>
<td>calcium</td>
<td>15</td>
</tr>
<tr>
<td>Cd</td>
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<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
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<td>Cu</td>
<td>copper</td>
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</tr>
<tr>
<td>DEM</td>
<td>digital elevation model</td>
<td>39</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
<td>8</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
<td>48</td>
</tr>
<tr>
<td>Fe</td>
<td>iron</td>
<td>18</td>
</tr>
<tr>
<td>GPS</td>
<td>global positioning system</td>
<td>40</td>
</tr>
<tr>
<td>K</td>
<td>potassium</td>
<td>15</td>
</tr>
<tr>
<td>k</td>
<td>exponential decay constant; litter mass loss rate</td>
<td>49</td>
</tr>
<tr>
<td>Mg</td>
<td>magnesium</td>
<td>15</td>
</tr>
<tr>
<td>mg/l</td>
<td>oxygen concentration, milligram per litre</td>
<td>41</td>
</tr>
<tr>
<td>Mn</td>
<td>manganese</td>
<td>18</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
<td>15</td>
</tr>
<tr>
<td>°</td>
<td>slope, in degrees from horizontal</td>
<td>57</td>
</tr>
<tr>
<td>°C</td>
<td>temperature, degrees Celsius</td>
<td>41</td>
</tr>
<tr>
<td>P</td>
<td>phosphorus</td>
<td>15</td>
</tr>
<tr>
<td>p</td>
<td>significance value</td>
<td>61</td>
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<tr>
<td>Pools km⁻²</td>
<td>vernal pools per km squared</td>
<td>57</td>
</tr>
<tr>
<td>R²</td>
<td>coefficient of determination, proportion of variation explained by model</td>
<td>61</td>
</tr>
<tr>
<td>S</td>
<td>sulfur</td>
<td>15</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
<td>49</td>
</tr>
<tr>
<td>t</td>
<td>time (days)</td>
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</tr>
<tr>
<td>UTM</td>
<td>universal transverse Mercator coordinate system</td>
<td>38</td>
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<tr>
<td>Zn</td>
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CHAPTER ONE
LITERATURE REVIEW

1.1 Introduction

Soils of high latitude forests, which hold approximately 59% of worldwide soil carbon stocks, represent a significant potential carbon sink (Dixon et al., 1994). The rate of carbon capture through primary production currently exceeds the rate of carbon loss via decomposition in these ecosystems (Dixon et al., 1994; Krebs, 2001; Lal, 2005). In order to adequately understand carbon flux at large scales, the fine-scale variables that influence carbon cycling, including decomposition must be more clearly understood (Dixon et al., 1994; Malhi et al., 1999; Coleman et al., 2004; Fang et al., 2007). The decomposition of plant-based organic matter is regulated by meteorological and hydrological factors (i.e. moisture), decomposer community, and substrate physicochemical characteristics (Malhi et al., 1999; Coleman et al., 2004; Boon, 2006). As the direct effect of climate change will be shifts in precipitation and weather patterns, a greater understanding of the influence of environmental factors on the process of decomposition at fine scales must be gained in order to determine the potential effect of climate change on soil carbon fluxes.

Climatic factors, such as temperature and precipitation, are rarely measured at local scales, so a fine-scale correlate, such as soil moisture, is typically monitored and linked to larger climatic patterns. Soil moisture influences decomposition directly, through the water requirements of decomposer organisms, and indirectly, through the control of oxygen availability (Aerts, 1997). Where oxygen is abundant, such as upland forest floors, moisture availability is often the limiting factor for decomposition (Rey et al.,
In contrast, in low-lying locations with abundant surface water oxygen is often the limiting factor for decomposition (Langhans and Tockner, 2006). Perennial high-moisture surface conditions where oxygen is limiting, such as in coniferous bogs, can lead to decomposition rates lower than adjacent in upland, leading to long term organic carbon storage (Willoughby, 1974; Yurova and Lankreijer, 2007). Highly oxygenated surface water environments, such as streams and floodplains, have been shown to have decomposition rates higher than the surrounding upland and contribute little to organic carbon storage (Ellis et al., 1999; Langhans and Tockner, 2006). Vernal pools, a form of seasonally inundated wetlands found throughout North Eastern North America, have been proposed as a potential ‘hot spot’ of decomposition due to high moisture and oxygen availability; however, this claim remains largely untested (Barlocher et al., 1978; Battle and Golladay, 2001; Ball et al., 2008).

Vernal pools occur in small, shallow depressions in the forest floor (Zedler, 2003) and are characterized by a surface area of less than 1 ha, high invertebrate diversity, and lack of fish (Brooks, 2000; Colburn, 2004). The primary energy source in most pools is dead organic matter, in the form of leaf litter from surrounding uplands (Palik and Batzer, 2006; Wetzel, 2006). The effect of non-permanent surface water on decomposition within the forest floor is varied, with frequency of re-inundation increasing litter breakdown rates in isolated seasonal wetlands and length of time flooded increasing decomposition in riparian floodplains (Willoughby, 1974; Battle and Golladay, 2001; Langhans and Tockner, 2006). Comparison of decomposition within vernal pools to decomposition within non-flooding upland requires a more detailed understanding of both the vernal pool ecosystem and litter decomposition.
1.2 Vernal Pool Structure and Function

Seasonal forested wetlands known by a variety of names, including vernal pools (Colburn, 2004), seasonal forest ponds (Brooks, 2000), and cryptic wetlands (Lindsay and Creed, 2005) are found throughout the forests of northeastern North America. These hydrologically temporary and geographically isolated wetlands are distinguished from other wetlands by their geography, hydrology, and biological communities (Colburn, 2004). Vernal pools are found within a forested matrix (Colburn, 2004; Brooks, 2005), and are often undetectable under the forest canopy by conventional aerial or satellite imagery (Creed et al., 2003). They occur in small, shallow depressions in the forest floor (Zedler, 2003) typically less than 1 m deep (Colburn, 2004) and less than 1 ha in area (Brooks, 2005). Vernal pool hydrology is dominated by seasonal filling, with maximum water depth usually reached in early spring as a result of snow melt. Dry-down typically occurs by mid to late summer and is followed by re-filling in the autumn (Colburn, 2004). By definition vernal pools have no permanent overland hydrological connection (Brooks, 2005). Vernal pools are also homes to a distinct assemblage of vertebrate (Colburn, 2004) protist and invertebrate (Brooks, 2000) species that have adapted to survive dry periods by migrating, transforming, or withstanding temporary desiccation (Batzer and Wissinger, 1996).

Vernal pools serve a variety of important functions in the ecosystem, where they influence processes, such as the water cycling (Brooks, 2005), decomposition (Barlocher et al., 1978), and nutrient cycling (Creed et al., 2003). Many questions, however, still remain unanswered about the geography, hydrology, and biology of vernal pools, as well as their role in ecological functioning (Colburn, 2004).
1.2.1 Geography

Vernal pools are found throughout the recently glaciated north eastern part of North America where the combination of glacial landforms (Palik et al., 2003), temperate climate (Brooks, 2004), and dominant forest biome (Calhoun and DeMaynadier, 2008) leads to the formation of these temporary wetlands across the landscape. Vernal pools in this area share greater similarity with respect to geographical constraints, such as glacial landforms, geomorphology, and surrounding forest ecosystem (Swanson et al., 1988; Rheinhardt and Hollands, 2008), than with temporary wetlands elsewhere. A limited set of geographical constraints can also be used to develop methodologies and models to locate vernal pools within this landscape. As a result it is reasonable to treat them as a single, albeit diverse, habitat type (Calhoun and DeMaynadier, 2008).

Abiotic geographic features such as surficial geology and topography constrain the location of vernal pools by their effect on surface permeability and prevalence of small topographic depressions (Palik et al., 2003; Rheinhardt and Hollands, 2008). The differences characterize three broad regions of northeastern North America which differ in the location and distribution of vernal pools (Rheinhardt and Hollands, 2008). The most northerly region, the Canadian Shield, stretching south to northern Michigan and southern Ontario, contains vernal pools located predominantly within local depressions underlain by pre-Cambrian igneous and metamorphic bedrock (Rheinhardt and Hollands, 2008). This study was carried out on vernal pools in the Canadian Shield region. Within the New England Igneous region, extending southeast of the Canadian Shield from central New York east to New England, vernal pools are found sporadically where low permeability loess deposits occur on till or bedrock at medium to high elevations (Grant,
Finally, in the Great Lakes Sedimentary region extending southwest of the Canadian Shield from south central Wisconsin to central New York, vernal pools tend to occur in the small basins found in the kettle topography of ground or end moraines, as well as in association with larger wetland complexes (Rheinhardt and Hollands, 2008). The topography immediately surrounding vernal pools tends to be relatively flat, with 95% of pools located in a Massachusetts survey occurring on slopes of less than 9.3° within 10m of the pool locations (Grant, 2005).

Geographical constraints on vernal pool occurrence can also be used to identify potential vernal pool locations, through the application of relevant abiotic and biotic conditions such as average slope, forest type and soil type as criteria for both manual identification methods, such as field surveys (Creed et al., 2003) and photographic interpretation (Lathrop et al., 2005), and automated identification methods, such as geospatial modeling (Grant, 2005). Elevation, surficial geology, and perennial water features have been used to aid visual identification of vernal pools on digital orthophotography (Lathrop et al., 2005). Slope has been found to be a good predictor of vernal pool locations and boundaries across geologic regions (Lindsay et al., 2004; Grant, 2005), and has been utilized to direct field identification of vernal pools within Ontario (Creed 2003; Appendix 1). Surficial geology, distance to perennial water features, and density of anthropogenic settlements (Grant, 2005) as well as glacial landform and upland forest type (Palik et al., 2003) have been used to refine predicted vernal pool locations.

1.2.2 Overstory

The upland forest matrix has a greater influence on vernal pools than larger, permanent wetlands, due to the higher proportion of edge to interior habitat within vernal pools.
(Palik and Batzer, 2006). Upland surrounding a vernal pool can influence pool processes and structures by providing energy input via leaf litter deposition (Palik and Batzer, 2006; Tank et al., 2010), regulating water levels (Colburn, 2004), and creating diverse wildlife habitat (DeMaynadier and Hunter, 1995; Windmiller, 1996). Leaf litter from the surrounding upland areas has been found to account for 71% of annual litter input to vernal pools of Minnesota (Palik and Batzer, 2006). Upland litter is of lower quality than wetland litter, therefore the high proportion of upland litter leads to slower litter decomposition rates in vernal pools than larger wetlands (Skelly et al., 2002; Palik and Batzer, 2006).

The presence of trees on the margins of vernal pools partially regulates dry-down rates through the opposing effects of reducing water temperatures and uptake of soil water. Trees at pool margins shade the water, keeping water temperatures low and preventing direct evaporation (Skelly et al., 2002). Adjacent trees also lower the surrounding water table and accelerate dry-down through root uptake and evapotranspiration (Halverson et al., 2003; Brooks, 2004; Semlitsch and Skelly, 2008). The adjacent upland areas also provide essential forage and breeding habitat (Leibowitz and Brooks, 2008) for migratory vertebrate species such as salamanders (DeMaynadier and Hunter, 1995; Rothermel and Semlitsch, 2002) and invertebrate species such as beetles and midges (Batzer and Wissinger, 1996; Merritt and Cummins, 1996).

1.2.3 Hydrology

The unique hydrology of vernal pool results in a duality of wet and dry states that strongly influence the biotic and abiotic processes occurring within and surrounding the pools. The typical hydrological regime of a vernal pool involves filling beginning in late
fall or early spring, reaching a maximum level in early to mid-spring, followed by a gradual drop in water levels until a “basin-dry state” is reached in late summer (Leibowitz and Brooks, 2008). The vernal pool hydroregime is characterized for different sites and years in terms of hydroperiod, which is defined as the length of time the pool contains standing water each year (Leibowitz and Brooks, 2008). The timing of pool filling is also an important variable. Precipitation driven pools are typically filled during the spring and have short hydroperiods responsive to short-term precipitation balance, whereas pools filled by groundwater typically fill in the fall or spring and have longer hydroperiods responsive to long-term subsurface hydrological conditions (Rheinhardt and Hollands, 2008).

Water levels for a given pool at any specified point in time and location are determined by a balance of water inflow and outflow due to precipitation, groundwater, overland flow, and evapotranspiration (Brooks, 2005; Leibowitz and Brooks, 2008). Precipitation is usually a major input of water to vernal pools, either directly through interception or indirectly through overland flow during rainfall events (Leibowitz and Brooks, 2008). In vernal pools monitored in Massachusetts rainfall accounted for more than 50% of the observed variation in water levels (Brooks, 2004). Groundwater can also be a major source of water inflow and outflow within vernal pools (Winter and LaBaugh, 2003; Leibowitz and Brooks, 2008) and is largely influenced by a combination of topography, geology, and climate. Groundwater is most important where subsurface flow is strong (Rains et al., 2006), soils are permeable to water (Gay, 1998; Winter and LaBaugh, 2003), and the pool volume to perimeter ratio is low (Phillips and Shedlock, 1993). Surface water flow into or out of vernal pools is generally assumed to be minimal.
(Brooks, 2005), except when soil sediments are saturated (Leibowitz, 2003; Rheinhardt and Hollands, 2008). These conditions exist during spring snowmelt and high intensity precipitation events (Rheinhardt and Hollands, 2008), with greater overland outflow occurring from low-capacity pools. Evapotranspiration, the loss of water can occur directly from the pool surface or indirectly through root uptake and transpiration from the leaves of surrounding trees (Leibowitz and Brooks, 2008). Evapotranspiration is demonstrably the primary mechanism of water loss from vernal pools (Brooks, 2004, 2005). Evapotranspiration rates fluctuate seasonally since they are strongly influenced by temperature and shade (Leibowitz and Brooks, 2008).

Vernal pools function within the regional hydrological regime in a manner similar to permanent wetlands, transiently storing and releasing groundwater and precipitation (Bullock and Acreman, 2003). The effect of vernal pools on runoff from a catchment basin is primarily influenced by the saturation state both of local wetlands and drainage area (Verry and Kolka, 2003; Lindsay et al., 2004). The degree of hydrological connectivity between pools pool basin morphology also influence catchment runoff (Lindsay et al., 2004). During dry periods pools serve as water sinks (Quinton and Roulet, 1998; Metcalfe and Buttle, 1999), decreasing overall flow from a drainage basin (Lindsay et al., 2004). During wet periods pools serve as water moderators (Glenn and Woo, 1997; Quinton and Roulet, 1998), decreasing maximum flood peak and increasing duration of flow (Lindsay et al., 2004). Lindsay et al. (2004) found that runoff from landscapes dominated by vernal pools was better predicted by wetland metrics such as total wetland area or volume than more commonly used basin metrics such as catchment area and slope.
1.2.4 Community Composition and Biodiversity

Biological communities within vernal pools are comprised of a diverse and unique combination of plant, bacteria, fungi, invertebrate, and vertebrate species, that can adapt to annual pool filling and drying. The composition of both invertebrates (Colburn et al., 2008), and vertebrates (Semlitsch and Skelly, 2008) is dependent on hydrology, water chemistry, and surrounding landscape. Species present can include aquatic, terrestrial, and amphibious species (Joyal et al., 2001; Colburn, 2004; Skidds and Golet, 2005; Calhoun and DeMaynadier, 2008; Colburn et al., 2008; Semlitsch and Skelly, 2008). The lack of permanent standing water and surface connections to permanent bodies of water precludes fish, however, which constitute the top predators in many freshwater aquatic ecosystems (Colburn, 2004).

Vernal pools are used by biotic organisms for a variety of purposes, including forage, refuge, and breeding. Individual species life histories span a continuum of dependency, from obligatory in the case of wood frogs (Calhoun and DeMaynadier, 2008) and fairy shrimp (Colburn, 2004), to facultative in the case of caddisfly and mosquito larvae (Colburn, 2004), to infrequent use by woodland birds and mammals (Mitchell et al., 2008). Heterogeneity of habitat and annual disturbance in vernal pools leads to disproportionately high biodiversity within, and between pools (α and β diversity, respectively). This suggests that vernal pools should be potential hot spots of biodiversity and genetic diversity within the landscape (Colburn, 2004; Ball et al., 2008).

The composition of the plant community within and immediately surrounding vernal pools depends on biogeographic region and the physical characteristics of each pool (Cutko and Rawinski, 2008). The plant species occurring in any given vernal pool is
highly dependent on the composition of regional vegetation, which in turn is determined by climate and biophysical characteristics (Cutko and Rawinski, 2008). Percent similarity between plant communities in New England vernal pools has been found to decrease over increasing geographical distance (Cutko and Rawinski, 2008). The physical distribution of plant species within a vernal pool can be divided into central, overarch, and perimeter zones within the pool basin reflecting species tolerance of flooding and shading (Colburn, 2004; Rheinhardt and Hollands, 2008). The central zone, with the deepest water levels, longest hydroperiod, and most open canopy, is the most likely to contain aquatic plants (Baldwin and Mitchell, 2000), along with temporary algal mats (Colburn, 2004). The overarch zone, which has a short-hydroperiod, shallow water, and variable shade is characterized by a relatively sparse flowering plant community of facultative wetland species (Colburn, 2004; Cutko and Rawinski, 2008). The perimeter zone, which has little standing water and the highest level of shading is dominated by flood-tolerant tree and shrub species such as red maple, eastern hemlock, and alders, species that differ significantly from the composition of the surrounding forest (Colburn, 2004).

Micro flora and fauna populations in vernal pools include a wide variety of bacteria, protozoa, algae, and fungi (Colburn, 2004). These microbes outnumber both invertebrates and vertebrates in species richness and number of individuals (Colburn, 2004). Bacteria and fungi, as in terrestrial and stream habitats, play a key role in the breakdown of leaf litter in vernal pools (Kirk and Farrell, 1987; Colburn, 2004; Nikolcheva and Barlocher, 2004). Bacteria are present and active in both aquatic and terrestrial phases of vernal pools. They release nutrients from the leaf litter and provide
forage for protazoa and insects (Laird, 1988). Fungi species composition and biomass within vernal pools varies over time depending on hydroregime. During the dry phase, terrestrial fungi which require abundant oxygen predominate (Colburn, 2004). Upon inundation, aero-aquatic fungi which have both aquatic and terrestrial stages to their life cycle colonize litter within vernal pools (Colburn, 2004; Wang et al., 2005). During inundation, aquatic hyphomycetes (Ingoldian fungi) occur at low densities in vernal pools (Colburn, 2004), despite being the dominant decomposer fungi in streams (Suberkropp and Klug, 1980; Webster and Benfield, 1986; Nikolcheva and Barlocher, 2004). Permanent or semi-permanent flooding within Pennsylvania vernal pools has been found to lead to higher fungal and bacterial biomass on litter (Inkley and Wissinger, 2008). Drying or freezing of litter prior to inundation, which is more likely to happen with later filling dates, has been shown to increase fungal biomass up to 3 months after inundation (Barlocher, 1992). More research is needed on the micro flora and fauna of vernal pools in order to understand their diversity and function within vernal pools.

Vernal pool invertebrates are a diverse group including numerous aquatic species. Typical vernal pool invertebrates include various aquatic insect larvae such as caddisflies (Trichoptera), dragonflies and damselflies (Odonata), as well as midges (Diptera: Chironomidae, Chaoboridae, Corethrellidae), mosquitoes (Diptera: Culicidae) and aquatic crustaceans (fairy shrimp, finger clams, amphipods, water fleas, ostracods and copepods) (Eriksen and Belk, 1999; Colburn et al., 2008). In addition collemmbola, segmented worms, gastropods, and arachnids are often present (Williams, 2006).
Invertebrate taxon richness and diversity is positively related to vernal pool hydroperiod. Invertebrate community complexity increases with extended hydroperiods (Schneider and Frost, 1996; Brooks and Doyle, 2001). Longer hydroperiods allow a shift in community structuring forces from competition for resources in short hydroperiod pools to predation by insect and amphibian larvae in long hydroperiod pools (Colburn, 2004; Williams, 2006). Invertebrate species composition can also be affected by topography, geology, and surrounding vegetation (Merritt and Cummins, 1996). These factors influence water temperature, pH, and dissolved oxygen content, as well as freezing depth in winter. Species not adapted to survive large variations in these conditions will generally be excluded from vernal pools (Williams, 1987).

The most-studied group within vernal pools are obligate and facultative pool-breeding amphibians such as the Jefferson salamander (*Ambystoma jeffersonianum*) and Spotted salamander (*A. maculatum*) (Colburn, 2004; Ball et al., 2008). Pool-breeding amphibians use the wet-phase of vernal pools to support larval stage (Colburn, 2004). Amphibian species composition within each pool is determined by hydroperiod, which limits presence of particular species based on minimum length of larval stage (Skidds and Golet, 2005; Semlitsch and Skelly, 2008). Hydroperiod has also been positively related to both richness and abundance of amphibian species (Pechmann et al., 1989; Baldwin et al., 2006). Pool volume, which is correlated to hydroperiod (Brooks and Hayashi, 2002), has been positively associated with breeding effort by wood frogs (*Rana sylvatica*) and spotted salamanders (*A. maculatum*) (Rowe and Dunson, 1993). The surrounding forest canopy can also influence the rate of larval development through the effects of shade on water temperatures (Skelly et al., 2002).
Other species such as turtles, snakes, birds and mammals which typically do not breed within vernal pools make use of pools directly, through forage and refuge, and indirectly, through consumption of insects and other prey which migrate out of the pool upon drying (DeGraaf and Yamasaki, 2001). Turtles utilize vernal pools as stop-over locations when traveling from wetland to nesting habitat, as well as to forage, bask, and mate (Joyal et al., 2000, 2001). Turtles found within vernal pools include specialists such as the spotted turtle (*Clemmys guttata*), Blanding’s turtle (*Emydoidea blandingii*), and wood turtle (*Glyptemys insculpta*) (Joyal et al., 2001; Compton et al., 2002). Generalists such as the snapping turtle (*Chelydra serpentia*) and painted turtle (*Chrysemys picta*) have also been found in vernal pools (Colburn, 2004). Snakes (Ernst and Ernst, 2003), birds (Scheffers et al., 2006), and mammals (Whitaker and Hamilton, 1998) tend to be transient users of vernal pools. These species take advantage of the proliferation of vegetation, invertebrates, amphibians during the flood and dry-down stages through feeding in and around vernal pools (Mitchell et al., 2008).

1.2.5 Conservation and Management

Vernal pools are important to the broader ecosystems in which they are found due to their unique hydrology and community composition (Colburn, 2004). Vernal pools are crucial in the function of forest ecosystems, by serving as outflow regulators in headwater areas (Creed et al., 2003), and critical in maintaining local biodiversity (Semlitsch and Bodie, 1998). Small ponds and pools may also serve as useful model ecosystems for ecological research and environmental education (De Meester et al., 2005; Gruner and Haley, 2008).
Vernal pools are protected by various legislation, regulations, and guidelines enacted at numerous governmental levels (Colburn, 2004; Mahaney and Klemens, 2008). Within Ontario, vernal pools are not specifically protected by any legislation or regulations. However, all wetlands on Canadian Federal lands, including vernal pools, are protected from any ‘net loss of function’ (Mahaney and Klemens, 2008). Within the United States, the Clean Water Act protects all navigable waters and their tributaries from loss of chemical, physical, or biological integrity (Downing et al., 2003; Mahaney and Klemens, 2008). Isolated wetlands which are non-navigable are also protected under this legislation if they are demonstrated to have a 'significant nexus' to navigable waters and contribute to the integrity of these protected waters (Downing et al., 2003). Several states have regulations specifically to protect vernal pools including Maine, Massachusetts, Connecticut, and New Jersey (Christie and Hausmann, 2003; Mahaney and Klemens, 2008).

Despite regional and national regulations, vernal pools are still threatened by pressures such as climate change, certain forestry practices, and land use changes. Climate change threatens vernal pools through changes in precipitation patterns. Predicted changes to weather patterns are likely to result in decreased hydroperiod, with earlier dry-down in spring and later pool filling in autumn (Brooks, 2009). Forestry operations surrounding vernal pools influence pools directly through alterations in basin morphology and indirectly through changes in hydrology, canopy cover and litter fall patterns (Calhoun and DeMaynadier, 2008). Vernal pools can be affected by changes to surrounding upland through altered hydrological and habitat connectivity (Windmiller and Calhoun, 2008). Overall, many factors still threaten vernal pools, however legislation addressing these
important ecosystems specifically, driven by science and public opinion, is being pursued in an increasing number of jurisdictions (Grant, 2005; Lathrop et al., 2005).

1.3 **Leaf Litter Decomposition**

The breakdown of dead organic matter into its component parts, known as decomposition, is accomplished through a complex suite of chemical, physical, biological processes (Aerts, 1997; Berg, 2003). As organic matter decomposes, energy, CO₂, and nutrients are released into the environment (Berg, 2003). The remaining organic carbon is transformed into increasingly stable forms, such as humus and soil organic matter (Berg, 2003). Decomposition is a key determinant of nutrient and energy cycling rates in terrestrial ecosystems (Aerts, 1997). Understanding decomposition, including its rate, active processes, and influencing conditions, is important to our understanding of terrestrial ecosystems as a whole.

Within temperate forest ecosystems, leaf litter is a major component of dead organic matter input to the forest floor (Dickinson, 1974). Mass loss from leaf litter typically follows a negative exponential curve, with rapid initial loss, followed by decreasing rate of loss until mass loss approaches zero (Figure 1) (Aerts, 1997; Berg, 2003). Decomposition of leaf litter is accomplished through 3 simultaneous processes: leaching of soluble substances (Tukey, 1970), physical fragmentation of litter mass (Rubino et al., 2010), and biochemical oxidation of organic matter (Seastedt, 1984; Valiela et al., 1985; Webster and Benfield, 1986). Each process affects different organic compounds and nutrients within the litter, and is influenced by abiotic and biotic conditions within the litter environment (Witkamp, 1966; Berg et al., 1993; Berg, 2003).
Figure 1. Example decay curve of litter mass loss over time

Generalized negative exponential curve of litter mass loss over time during decomposition (Rovira and Rovira, 2010).
1.3.1 Litter Changes Over Time

1.3.1.1 Initial Litter Properties

The initial chemical composition of leaf litter is a complex mixture of organic and inorganic compounds (Jensen, 1974; Coleman et al., 2004). Litter chemistry determines the quality of a litter substrate, therefore the vulnerability of that litter to decomposition (Chapin et al., 2002; Hoorens et al., 2002). Litter quality significantly influences mass loss rate of a substrate (Hoorens et al., 2002). Although mechanisms of influence are still poorly understood, specific litter quality measures, such as carbon (C), nitrogen (N), carbon to nitrogen ratio (C:N), and lignin to nitrogen ratio (lignin:N), can be used as predictors of litter decomposition rate (Gallardo and Merion, 1992; Trofymow et al., 1995; Chapin et al., 2002). Litter quality, therefore chemical composition, varies between tissue type, species and ecosystems (Johansson, 1995; Aerts, 1997).

Carbon is the single largest elemental constituent in leaf litter, with a mean initial total carbon content of 50% in deciduous litter (Gillon et al., 1994; Ibrahima et al., 1995; Trofymow et al., 1995; Rubino et al., 2009). Evergreen litter has slightly higher total carbon content than deciduous litter (Trofymow et al., 1995). The organic carbon content within litter is composed of compounds of differing ease of decomposition. Labile metabolic compounds, such as sugars and amino acids, are lost rapidly from litter (Kirk and Obst, 1988; Trofymow et al., 1995; Chapin et al., 2002). Moderately labile structural compounds, such as cellulose and hemicellulose, are lost more slowly from litter (Trofymow et al., 1995; Chapin et al., 2002). Recalcitrant structural compounds, such as lignin and cutin, are lost very slowly from litter (Kirk and Obst, 1988; Trofymow et al., 1995; Chapin et al., 2002). The proportions of different organic carbon compound within
leaf litter contributes to the degree of litter quality. Litters high in labile compounds exhibit higher rates of mass loss (Chapin et al., 2002). Litters high in lignin and other recalcitrant compounds, such as evergreen litter, exhibit lower rates of mass loss (Trofymow et al., 1995; Chapin et al., 2002). Inorganic mineral content, measured as the ash remaining after combustion of leaf litter, tends to be greater in higher quality litter (Jensen, 1974; Trofymow et al., 1995).

Nitrogen (N) content ranges from 0.54% to 1.97%, with a typical mean of 0.75% (Beauchamp and Kelly, 1987; Gillon et al., 1994; Ibrahima et al., 1995; Gholz et al., 2000; Rubino et al., 2009). Carbon to nitrogen ratio (C:N) is a common measurement of initial litter quality (Gallardo and Merion, 1992), as lower C:N typically leads to more rapid decomposition.

Phosphorus (P) shows greater variability than C or N, with typical values of 0.075% (Beauchamp and Kelly, 1987; Parsons et al., 1990; Gillon et al., 1994; Trofymow et al., 1995). Other macronutrients such as calcium (Ca), potassium (K), magnesium (Mg), and sulfur (S) are tested for infrequently (Beauchamp and Kelly, 1987; Trofymow et al., 1995). The macronutrients of N, Ca, P, and Mg tend to differ the most between deciduous and evergreen leaf litter (Trofymow et al., 1995).

1.3.1.2 Early Decomposition

The early stage of decomposition within temperate ecosystems typically occurs in the first 2 to 3 weeks. This stage is characterized by rapid loss of mass, carbon, nutrients, and water soluble organic carbon compounds (Tukey, 1970; Davis et al., 2003). This loss is accomplished primarily through leaching, the dissolution and transportation of water-soluble substances (Tukey, 1970). Within the first 24 hours of soaking leaf litter can lose
up to 23% of initial mass (Taylor and Parkinson, 1988), 30% of carbon (Davis et al., 2003), and 50% of sugars (Ibrahima et al., 1995). The amount of each constituent lost varied widely between species and studies. Overall mass loss due to leaching has been found to range from 0% (France et al., 1997) to 30.8% (Day, 1983), depending on species (France et al., 1997). Broadleaved species typically lose more mass than needle-leaved species (Taylor and Parkinson, 1988; Chapin et al., 2002). Elemental carbon loss is often proportional to mass loss from litter (Ibrahima et al., 1995), with loss between 3% (Ibrahima et al., 1995) and 30% (Davis et al., 2003) of total carbon. N, P, and K are lost rapidly within the first 24 hours of decomposition (Day, 1983; France et al., 1997; Davis et al., 2003). Mg and Ca are lost more slowly. K and Mg experience the most complete loss from leaching, 98.1% and 65.6% of original content, respectively (Day, 1983). After 14 days of decomposition, leaching-induced losses of mass, organic carbon, and nutrients approached zero (Ibrahima et al., 1995; France et al., 1997; Davis et al., 2003). The amount and rate of nutrient loss from leaf litter is dependent on environmental moisture conditions (Preston et al., 2000; Trofymow et al., 2002) and proportion of leachable substances within litter (Trofymow et al., 2002).

1.3.1.3 Mid Stage Decomposition

Mid-stage decomposition occurs between 3 weeks to 12 months of litter exposure (Trofymow et al., 2002; Berg, 2003). The rate of litter mass loss decreases over time (Lousier and Parkinson, 1976; Davis et al., 2003). This stage is characterized by a shift from abiotic mechanisms of litter change to biotic mechanisms (Davis et al., 2003; Rubino et al., 2009, 2010). Mass loss from leaf litter within temperate forests over the first 12 month of decomposition is typically 30% ± 10 of original dry litter mass (Lousier
and Parkinson, 1976; Johansson et al., 1995; Davis et al., 2003; Rubino et al., 2010).

However, the amount of mass lost within the mid-stage of decomposition is highly variable (Berg, 2003; Rubino et al., 2009). The largest mass loss rate is within the first 3 to 5 months, with decreasing mass loss rates thereafter (Lousier and Parkinson, 1976; Davis et al., 2003).

Carbon losses from litter are proportional to overall mass loss during mid-stage decomposition (Davis et al., 2003; Rubino et al., 2009). Total carbon content is typically 50% of remaining dry mass throughout mid-stage decomposition (Davis et al., 2003).

The relative content of cellulose in litter decreases due to bacterial and fungal metabolic activity (Dickinson and Pugh, 1974). The relative content of lignin increases over mid-stage decomposition due to a lower rate of mass loss than other organic carbon compounds (Dickinson and Pugh, 1974).

The amount of nitrogen within leaf litter increases rapidly during the first month of exposure (Davis et al., 2003). Nitrogen content then increases slowly until an optimal C:N ratio is reached, typically between 20:1 and 30:1 (Lousier and Parkinson, 1978). Nitrogen increases are driven by retention of existing nitrogen and translocation of nitrogen from surrounding environments by fungal hyphae (Lousier and Parkinson, 1978; Coleman et al., 2004). Nitrogen concentration increases throughout the first 12 months of decomposition due to simultaneous increases in nitrogen content, and decreases in litter mass (Melillo et al., 1982; Davis et al., 2003; Rubino et al., 2010).

Other nutrients, including P, Ca, Mn, Fe, Zn, Mg, have been observed to increase in concentration over time (Lousier and Parkinson, 1978), however changes in amount and trends over time vary among litter species and study sites.
1.3.1.4 Late Stage Decomposition

Late stage decomposition is characterized by extremely slow mass loss (Berg, 2003). Mass loss observed after up to 6 years of decomposition ranged from 40% to 70% (Lousier and Parkinson, 1976; Trofymow et al., 2002; Berg, 2003). Late stage decomposition rates and mass at steady-state are driven by nutrient availability, in both leaf litter and surrounding environment, as well as microclimate, such as temperature and moisture (Berg et al., 1999; Trofymow et al., 2002).

Carbon loss is proportional to mass loss, therefore concentration remains constant (Lousier and Parkinson, 1978). A study of the decomposition of scots pine (Pinus sylvestris L.) litter measured lignin, cellulose and hemicellulose over 5 years (Berg et al., 1982). Lignin content was relatively stable for first the 2 years of decomposition, then declined slowly (Berg et al., 1982). Due to greater proportional mass loss, lignin concentration slowly increased over time (Berg et al., 1982). Cellulose increased in concentration over the first year of decomposition, then declined rapidly (Berg et al., 1982). Hemicellulose declined steadily throughout the 5 years of decomposition (Berg et al., 1982).

Litter retains most of its original nitrogen content up to 6 years of decomposition (Lousier and Parkinson, 1978; Trofymow et al., 2002). The carbon to nitrogen ratio was found to reach approximately 25:1 after 2 years of decomposition, and remain stable after up to 5 years of decomposition (Lousier and Parkinson, 1978). Other nutrients can increase, such as S and Na, fluctuate around a stable value, such as Ca and Mn, or decrease, such as K and Mg (Beauchamp and Kelly, 1987). The response of nutrients
was largely dependent on the nutrient availability in the leaf litter species and surrounding environment (Lousier and Parkinson, 1978).

In late stage decomposition leaf litter is also transformed into humus, a relatively recalcitrant organic matter which can persist in soils for long periods of time (Chapin et al., 2002). Slow decomposition of humus allows carbon and nitrogen to accumulate in the soil environment, to be released slowly over time or in larger quantities after a disturbance (Chapin et al., 2002). The specific chemical properties of humus varies between ecosystems, as it depends primarily on the litter substrate species (Chapin et al., 2002).

1.3.2 Litter Decomposition Processes

1.3.2.1 Leaching

Leaching is a primarily abiotic process where water soluble materials removed from leaf litter, with rate and completeness of loss influenced by litter chemistry and environmental conditions. Leaching is one of the first processes to act upon newly shed litter and can account for the loss of up to 30% of initial mass (Tukey, 1970; Day, 1982). Through leaching, water soluble substances within leaf litter such as Ca, Mg, carbohydrates, and phenolics (Barlocher, 1992) are dissolved into aqueous solution via precipitation, fog, standing, or flowing water (Tukey, 1970). Lignin and cellulose content remain unchanged through the initial leaching process (Ibrahima et al., 1995).

Environmental moisture content, including soil moisture (Rey et al., 2005), precipitation (Tukey, 1970), and standing water (Lockaby et al., 1996), is the primary environmental control of leaching loss rate. Moisture added after a dry period has been shown to disproportionately, but temporarily, increase leaching loss rates of mass, carbon, and
nitrogen (Taylor and Parkinson, 1988; Jarvis et al., 2007). Environmental conditions which increase energy in the leaf litter layer, such as temperature (Tukey, 1970; Rey et al., 2005) and light intensity (Tukey, 1970), have also been shown to have a positive relationship with leaching rate. Factors which affect the ability of water to enter the leaf litter, such as presence of a waxy cuticle (Harris and Safford, 1996), drying (Harrison and Mann, 1975), or freezing (Barlocher, 1992) can also influence leaching rates. Freezing, and subsequent thawing can lead to expansion of water in litter resulting in physical fragmentation (Barlocher, 1992; Wu et al., 2010). These physical changes increase mass and carbon (Harris and Safford, 1996) losses due to leaching. This loss accounts for 64.5% of first-year mass loss (Wu et al., 2010) and increasing carbon leached from leaf litter by 3.5 times (Harris and Safford, 1996).

1.3.2.2 Physical Breakdown

Whole litter is altered physically through fragmentation, the breakdown of litter into smaller pieces, and comminution, the mixing of leaf litter fragments with humus (Seastedt, 1984). Physical breakdown of litter is accomplished through abiotic processes, such as freeze/thaw cycles (Barlocher, 1992; Harris and Safford, 1996; Freppaz et al., 2007) as well as biotic processes, such as microarthropod feeding (Seastedt, 1984).

Although there is great diversity in form and function of soil fauna, fungal feeding microarthropods such as mites and collembolans are important contributors to leaf litter decomposition (Seastedt, 1984). Arthropods feeding activities results in direct loss of mass and carbon, and alterations to nutrient content, through consumption and defecation of leaf litter material (Seastedt, 1984). Increased surface area due to arthropod feeding leads to indirect losses from litter, through increased microbial colonization of litter.
(Witkamp, 1969; Harrison and Mann, 1975; Seastedt, 1984; Webster and Benfield, 1986; González and Seastedt, 2001). Inclusion of microarthropods in leaf litter can account for 50.7% to 78.7% of mass loss (Coleman et al., 2004) and double the rate of mass loss for whole leaves (Harrison and Mann, 1975).

1.3.2.3 Biochemical Transformation

Biochemical transformation of leaf litter consists of oxidation of organic carbon fractions and mineralization of inorganic nutrients (Witkamp, 1966). These transformations are accomplished primarily by bacteria and fungi seeking to liberate energy and nutrients (González and Seastedt, 2001; Coleman et al., 2004). Chemical transformation of litter is influenced directly by the decomposer community and indirectly by decomposing environment and litter substrate (Witkamp, 1966; Dickinson and Pugh, 1974; Aerts, 1997; Coleman et al., 2004). The C and N content, as well as the carbon to nitrogen (C:N) ratio, after the first year of decomposition can indicate the type and activity of decomposer organism present (Manzoni et al., 2010). Litter loss rates have been found to be positively related to fungal and bacterial abundance as well as total respiration rate (Witkamp, 1966). Models predict that terrestrial decomposition rate and litter mass loss are positively related to soil microbial diversity (Ekschmitt et al., 2001; Loreau, 2001; Hättenschwiler et al., 2005). Experimental evidence, however, suggests mass loss is increased with decreased microbial diversity (Griffiths et al., 2000; Hättenschwiler et al., 2005). Other decomposition processes, including nitrification, denitrification, and methane oxidation were increased with decreased microbial diversity (Griffiths et al., 2000; Hättenschwiler et al., 2005).
Environmental factors, such as climate and site nutrition, can influence the abundance and distribution of both bacteria and fungi (Witkamp, 1966; Coleman et al., 2004). Litter temperature and exposure time been found to be significantly positively correlated with fungal abundance and total respiration rates (Witkamp, 1966). Nutrient rich sites tend to have higher abundance of bacteria (Dickinson and Pugh, 1974). Nutrient poor sites or recalcitrant litter tend to have higher abundance of fungi (Dickinson and Pugh, 1974). They utilize hyphal networks to translocate nutrients mineralized within litter across greater distances (Coleman et al., 2004). Specific fungi, such as white-rot fungi, are specialized to decompose recalcitrant leaf litter components, such as lignin (Aerts, 1997).

1.3.3 Conditions Influencing Litter Decomposition

The rate and pattern of decomposition is influenced hierarchically by climate, litter chemistry, and decomposer community (Akselsson et al., 2005). Influencing conditions which vary on broad scales, such as climate, constrain conditions which vary at finer scales, such as decomposer community (Johansson et al., 1995; Aerts, 1997).

Environmental conditions influence litter mass loss directly via temperature (Aerts, 1997), moisture (Tukey, 1970), and site nutrition (Berg, 2003), and indirectly through its influence on litter chemistry (Aerts, 1997) and decomposer community (Witkamp, 1966; Coleman et al., 2004). Litter chemistry determines the relative proportions of labile and recalcitrant organic carbon fractions, which decompose at different rates, as well as nutrient content of leaf litter, which influences activity rates of decomposer organisms (Aerts, 1997; Berg, 2003; Gartner and Cardon, 2004). Decomposer community composition mediates decomposition rate through activity rate of decomposer organisms (Aerts, 1997). Interactions occur between these three factors, resulting in both synergistic
and antagonistic effects on litter decomposition (Webster and Benfield, 1986; Aerts, 1997). In order to understand why decomposition varies over space and time, the mechanisms by which environment, decomposer community composition, and litter chemistry affect decomposition must be understood.

1.3.3.1 Environment

Regional climate, including broad patterns of temperature and precipitation, exerts strong direct and indirect control on decomposition processes (Aerts, 1997). Climate directly affects leaching, through moisture available for dissolution of water soluble compounds. Litter fragmentation and oxidation are directly influenced by temperature and moisture through controls on activity of decomposer organisms (Aerts, 1997). Climate exerts indirect control on decomposition processes through litter chemistry. Temperature and precipitation within a region determines tree species present, therefore litter quality and decomposition rates (Aerts, 1997; Madritch and Cardinale, 2007). There is also evidence of interaction effects between climate and other conditions influencing decomposition. The influence of lignin content on decomposition processes changes across climates (Meentemeyer, 1978; Johansson et al., 1995).

Climatic variables are rarely measured directly at time and place of study. The relationship between decomposition rate and climate is often determined using climatic analogues calculated from measurements taken at nearby climate stations (Berg et al., 1993; Johansson et al., 1995; Aerts, 1997; Trofymow et al., 2002). Long-term climatic averages, such as 30-year average annual temperature, annual precipitation, and summer precipitation, have been found to significantly predict leaf litter mass loss after 3 and 6 years, across 18 upland forest sites distributed throughout Canada (Trofymow et al.,
Actual evapotranspiration (AET), an index which integrates climatic energy and moisture availability in the litter layer (Thornthwaite et al., 1957; Meentemeyer, 1978; Aerts, 1997), has been observed to significantly predict first-year mass loss from leaf litter (Meentemeyer, 1978; Johansson et al., 1995; Aerts, 1997). AET has been observed to be a better predictor of first year mass loss rates than leaf litter chemistry, including lignin content and C:N ratio, at regional and global scales (Berg et al., 1993; Aerts, 1997). Due to low variability of AET within some climate regions and site-specific interactions between climate, litter quality, and decomposer community, litter chemistry is a better predictor of mass loss at sub-regional scales (Berg et al., 1993; Johansson et al., 1995; Aerts, 1997).

Site scale microclimate, similarly to regional climate, directly influences decomposition processes through temperature (Magid et al., 2004) and moisture (Harris and Safford, 1996; Rey et al., 2005) controls on metabolic rate of decomposer community. Air and soil temperature have been observed to be directly related to carbon mineralization rates between 3°C and 25°C (Magid et al., 2004; Hoffmann et al., 2010), with greater response to temperature above 20°C in low-lignin litter (Hoffmann et al., 2010). Soil water content has been found to be positively related to carbon mineralization rate (Rey et al., 2005). Above 80% soil water, however, decomposition was limited by low oxygen availability (Rey et al., 2005).

Leaf litter mass loss rates are influenced by availability of nutrients within the forest floor, including such as nitrogen and phosphorus (Aerts and de Caluwe, 1997), as well as minerals such as Cd, Zn, Cu (Berg, 2003). Leaf litter mass loss rates have been observed to increase on nutrient-rich sites (Aerts and de Caluwe, 1997; Berg, 2003), with greater
effect on deciduous leaf litter (Berg, 2003). At nutrient poor sites heavy metals such as Cd, Cu, and Zn have been observed to decrease the overall mass loss, however no relationship was found on a nutrient rich site (Berg, 2003). Mass loss rates have been observed to decrease at sites with higher nitrogen (Kaushik and Hynes, 1971; Berg, 2003; Hobbie, 2008). This effect was greater in leaf litter with high lignin content. This effect may be due to inhibition of lignin-degrading enzymes (Hobbie, 2005) or interaction between nitrogen and decomposer community (Hobbie, 2008). The relationship between leaf litter mass loss rates and nutrient availability is influenced by a complex interaction of litter chemistry, stage of decomposition, and climate. This interaction warrants further study in order to develop predictive relationships (Hobbie, 2008).

1.3.3.2 Substrate

At a global scale, leaf litter chemistry at the start of decomposition is a secondary predictor of mass loss rates and values throughout decomposition, after climate (Aerts, 1997; Berg, 2003; Gartner and Cardon, 2004). Variation of initial litter chemistry is determined by climate (Aerts, 1997), tree species and site nutrition (Berg, 2003). Organic carbon constituents within leaf litter influence leaf litter mass loss rates through relative proportions of labile and recalcitrant organic carbon fractions (Berg, 2003). Higher initial nutrient content within leaf litter leads to greater decomposition rates (Gosz et al., 1972; McClaugherty and Berg, 1987; Preston et al., 2000; Trofymow et al., 2002). Nitrogen has more complex relationship with mass loss rates and values (Aerts and de Caluwe, 1997; Berg et al., 1999; Hobbie, 2005).
Leaf litter mass loss rates in early stage decomposition have been observed to be related to water soluble organic carbon and lignin content within litter substrate (Hobbie, 2005). Lignin content in litter substrate has been negatively correlated with mass loss up to 1 year after litter exposure (Hobbie, 2005). The predictive power of this relationship is low in all experiments, and decreases with decreasing AET (Meentemeyer, 1978; Melillo et al., 1982; Aerts, 1997). The ratio of lignin to nitrogen has been demonstrated to be a significant secondary predictor of mass loss rates, after AET (Meentemeyer, 1978; Aerts, 1997). Initial Lignin to nitrogen ratio in litter was observed to have a negative relationship with mass loss rates (Meentemeyer, 1978; Melillo et al., 1982).

Higher initial nutrient content within leaf litter has been found to lead to greater decomposition rates in both early and late stages of decomposition (Gosz et al., 1972; McClaugherty and Berg, 1987; Preston et al., 2000; Trofymow et al., 2002). Nutrients required for metabolism of litter, such as P (Aerts and de Caluwe, 1997), Mn (Berg et al., 2010), Ca (Virzo De Santo et al., 2008), and N (Hobbie, 2008), can influence the growth of bacteria and fungi, therefore mass loss rates. Initial phosphorus (P) content within litter is positively related to early mass loss. P content within litter is easily lost due to leaching. Up to 65% of initial leaf litter P has been observed to be lost in first 4 days after litter exposure (Aerts and de Caluwe, 1997). Lack of available P may be limiting to biologically mediated decomposition. Initial litter manganese (Mn) content within leaf litter was positively correlated with accumulated mass loss up to 5 years of litter exposure. This indicates that Mn is potentially a key component in lignin degrading enzymes (Berg et al., 2010). Calcium (Ca) content within leaf litter after 1 year of exposure was positively related to decomposition rates after 3 years in both temperate
and boreal forests. Calcium allows fungal hyphae to develop hydrophobic coatings which protect them from microbial attack and increases their ability to metabolize leaf litter (Virzo De Santo et al., 2008). Higher N content in leaf litter has been associated with higher initial mass loss rates (Prescott, 1995; Hobbie, 2005) at low-nutrient sites when N is a limiting nutrient to decomposition (Aerts and de Caluwe, 1997). Nitrogen within litter substrate is also positively correlated with higher mass loss values (Berg et al., 1999). This is potentially due to a chemical interaction between nitrogen and lignin, which leads to the formation of more recalcitrant organic carbon compounds (Aerts, 1997).

1.3.3 Community

The decomposer community within leaf litter consists of bacteria and fungi, arthropods of various sizes, as well as even smaller organisms such as nematodes (Seastedt, 1984; Blair et al., 1990). This diverse community accomplishes both physical and chemical breakdown of leaf litter (Coleman et al., 2004). The decomposer community influences decomposition processes, as increased activity and population size leads to greater mass loss rates (Witkamp, 1966). The presence of specific species, such as white rot fungi which decompose lignin, can lead to more rapid or more complete decomposition (Aerts, 1997). The abundance, composition, and activity of a decomposer community are influenced primarily through litter substrate chemistry, and secondarily through regional climate (Swift et al., 1979).

The abundance, activity, and community composition of bacteria and fungi have been found to be positively related to mass loss rates (Witkamp, 1966; Parnas, 1975; McGuire and Treseder, 2010). Community composition has also been linked to the total mass loss
during decomposition (McGuire and Treseder, 2010; Fontaine et al., 2011). Abundance and respiration rate of both bacteria and fungi have been found to be positively correlated to rates of litter breakdown, but the degree of correlation was influenced by temperature, moisture, and stage of decomposition (Witkamp, 1966). Mass loss rates have been observed to decrease in the presence of ectomycorrhizal fungi (McGuire and Treseder, 2010), as it suppresses other heterotrophic decomposers (Gadgil and Gadgil, 1971). Species richness, irrespective of specific species present, has been found to be positively related to microbial metabolic activity (Salonius, 1981). Evidence suggests there is, however, an upper saturation limit of species richness effect (Wohl et al., 2004; Bell et al., 2005). Community composition of bacteria and fungi also determines the total mass loss during decomposition (Fontaine et al., 2011). Only certain species, such as white-rot fungi, can decay lignin completely (Aerts, 1997). Input of new litter can stimulate decomposition of recalcitrant fractions in previously decomposed litter by providing an additional energy source for specialized fungi (Fontaine et al., 2011).

The presence of arthropods within the forest floor layer has long been associated with increased mass loss rates and decreased standing stocks of leaf litter (Seastedt, 1984; Coleman et al., 2004). The relative contribution of arthropods to overall decomposition rates is influenced by litter composition (Blair et al., 1990; Kaneko and Salamanca, 1999) and climate (González and Seastedt, 2001). Arthropod presence has been observed to double decomposition rates and decrease leaf litter remaining after 1 year of decomposition to 58% (Seastedt and Crossley, 1984). This effect is potentially due increased surface area from fragmentation and increased palatability to bacteria and fungi through conditioning (González and Seastedt, 2001). Litter substrate species also
influences the influence of arthropods on litter mass loss. Arthropods have been found to contribute a greater proportion of overall litter mass loss when multiple litter species are present (Kaneko and Salamanca, 1999), or when lower-quality litter is present (Seastedt, 1984). The effect of arthropods on mass loss rates is also influenced by decomposition environment. Locations with high moisture and temperatures are more favourable to arthropod growth, increasing abundance and activity, therefore allowing fauna to account for a greater proportion of overall mass loss rates (González and Seastedt, 2001).

Previous research of mass losses from litterbags in both upland forests (Seastedt, 1984) and ephemeral streams (Mackay and Kersey, 1985) have reported higher litter mass loss in coarse mesh bags. This result has been attributed to the exclusion of macroinvertebrates from fine mesh litterbags (Seastedt, 1984). A review by Seastedt (1984) found leaf litter lost on average 23.1% greater mass per year in the presence of macroarthropods, than when they were excluded.

1.3.4 Decomposition Models

Modeling decomposition, including its progression, processes, and influencing conditions, is a crucial step in understanding the global carbon cycle (Adair et al., 2008). Decomposition models can predict how anthropogenic actions, such as climate change, may influence the uptake, release, and sequestration of carbon and other nutrients (Moorhead and Sinsabaugh, 2006). Ecosystem models simplify complex systems to a set of hypothetical state variables. Inputs, outputs, and potential interactions are represented through mathematical relationships (Manzoni and Porporato, 2009). Response models quantify the relationship between an ecosystem state variable, such as mass remaining (Gholz et al., 2000) or nitrogen content (Manzoni and Porporato, 2009), as well as time.
These models are interested only in the response of the ecosystem variable. Response models are validated against observation data, and generate metrics describing change over time which can be used to compare various conditions under which decomposition occurs (Aerts, 1997; Gholz et al., 2000). More complexity can be added to response models through multi-variable mathematical functions that serve as metrics to compare decomposition between sites, years, and studies (Gholz et al., 2000).

The negative exponential function is frequently used to model the fraction of litter mass remaining at successive time steps (Olson, 1963; Gholz et al., 2000). This model represents the 3 stages of decomposition and the observed decrease in mass loss rate over time. This model is used to calculate instantaneous decay rate, k, which represents the constant fraction of mass loss over time (Olson, 1963). The calculated k can then be used to compare decomposition rates between sites (Gholz et al., 2000), litter types (Lousier and Parkinson, 1976; Adair et al., 2008), and experimental methodology (Anderson, 1973; Cotrufo et al., 2010). The total value of leaf litter mass loss may be estimated by the inclusion of an asymptote within the model (Berg et al., 2010). The negative exponential models based on the assumption that net mass loss represents overall decomposition, therefore the sum of component functions of organic carbon fractions, which is not always valid (Minderman, 1968; Anderson, 1973).

The multiple exponential model has been found to improve fit of litter mass loss models. Mass loss from each organic fraction is represented by separate parameters (Minderman, 1968; Lousier and Parkinson, 1976; Bunnell et al., 1977; Beauchamp and Kelly, 1987; Gillon et al., 1994; Adair et al., 2008). The model parameters include proportion of mass remaining and instantaneous decay rates for each fraction included (Lousier and
Parkinson, 1976; Gillon et al., 1994; Adair et al., 2008). The fit of multiple exponential models varies between studies. This model performs much better than single-exponential in systems with low decomposition rates, multiple litter substrate species, and long-term studies (Lousier and Parkinson, 1976; Gillon et al., 1994; Adair et al., 2008). The negative exponential model, however, does not represent the changes of nutrients within leaf litter which do not change relative to mass loss, such as nitrogen, potassium, magnesium, and calcium (Lousier and Parkinson, 1978; Beauchamp and Kelly, 1987).

1.3.5 Litter Decomposition in Aquatic Environments

Freshwater aquatic environments, such as lakes, rivers, and wetlands, influence decomposition primarily through their effect on leaching and oxygen availability (Willoughby, 1974). Leaching of water soluble components of leaf litter occurs rapidly in when litter is submerged (Willoughby, 1974; Day, 1983; Davis et al., 2003). Near complete leaching has been observed between 3 and 25 days (Willoughby, 1974; Day, 1983; Davis et al., 2003). Although aquatic environments lead to rapid initial leaching rate, total mass loss due to leaching is similar between aquatic and terrestrial environments (Day, 1983; Corrigan, 2008). As with terrestrial decomposition, leaf litter species influences the rate and amount of mass loss due to leaching (Kaushik and Hynes, 1971; Willoughby, 1974; Wetzel, 2006). Deciduous species have been shown to lose greater litter mass to leaching than coniferous species (Tukey, 1970). The rate of bacterial and fungal breakdown of leaf litter within aquatic environments is highly dependent on the relationship between oxygen availability and oxygen demand (Willoughby, 1974). Decomposition rate is slowed by anaerobic conditions as oxygen is a limiting material (Blackburn and Petr, 1979; Neckles and Neill, 1994; Naiman et al.,
Hydrological properties of aquatic environments, such as water levels, source and annual cycles, can greatly affect moisture and oxygen regimes (Mitsch and Gosselink, 2000). Aquatic environments which maintain high oxygen, such as streams and riparia, show increased rates and amounts of mass loss (Mitsch and Gosselink, 2000). Aquatic environments which have low oxygen, such as peat lands and swamps, show decreased rates and amounts of litter mass loss (Mitsch and Gosselink, 2000).

### 1.3.6 Litter Decomposition in Vernal Pools

My survey of the vernal pool literature uncovered only two studies specifically examining decomposition of leaf litter within vernal pools (Barlocher et al., 1978; Palik and Batzer, 2006). Mass loss from leaf litter was initially rapid, likely due to leaching. This is similar to patterns observed in other aquatic environments (Mitsch and Gosselink, 2000). After 1 year, 52% to 67.7% mass was lost from Sugar Maple (*Acer saccharum* Marsh.) litter within vernal pools. Palik (2006) found that sugar maple leaf litter exhibited greater mass loss at pool margins, than at pool centres. This pattern indicates that decomposition within the centre of vernal pools may function similarly to low-oxygen aquatic environments, where mass loss rates are slowed.

Corresponding studies of decomposition have been carried out in other temporary wetlands, including swamps (Day, 1982; Battle and Golladay, 2001) and riparian zones (Langhans and Tockner, 2006). These studies found leaf litter mass loss between 49% and 85% after 1 year of litter exposure (Day, 1982; Shure et al., 1986; Conner and Day, 1991; Battle and Golladay, 2001). The presence of seasonal inundation increased litter mass loss rates compared to non-inundated and permanently inundated controls (Cuffney
and Wallace, 1987; Ryder and Horwitz, 1995; Lockaby et al., 1996; Kelley and Jack, 2002). Length of hydroperiod did not influence overall amount of mass loss or mass loss rate (Lockaby et al., 1996). Multiple flooding-drying cycles within an inundation period resulted in greater mass loss rates than single continuous flooding (Shure et al., 1986; Battle and Golladay, 2001).

Several studies have suggested that flooding patterns which increase oxygen availability lead to increased decomposition rates observed (Lockaby et al., 1996; Battle and Golladay, 2001). The oxygen content determines the path of microorganism metabolism, therefore the rate and completeness of litter decomposition (Lockaby et al., 1996; Baldwin and Mitchell, 2000; Battle and Golladay, 2001). Soils continuously inundated with stagnant or slowly flowing water have been observed to develop sustained anaerobic conditions (Neckles and Neill, 1994; Mitsch and Gosselink, 2000). In contrast, fluctuating inundation in soils increases organic matter mass loss, where each alternation of wet and dry periods results in a brief increase in decomposition rates due to increased oxygen availability (Birch, 1958; Palik and Batzer, 2006; Jarvis et al., 2007). Greater mass loss rates were reported for locations with multiple inundation-dry-down events, compared to those with a single inundation event, within temporary wetlands of Georgia, (Battle and Golladay, 2001), Minnesota (Palik and Batzer, 2006), and South Carolina (Shure et al., 1986). Fluctuating water levels also increased mass loss in seasonal wetlands (Shure et al., 1986; Glazebrook and Robertson, 1999; Battle and Golladay, 2001; Kelley and Jack, 2002; Palik and Batzer, 2006), compared to locations permanently inundated (Palik and Batzer, 2006) or dry (Shure et al., 1986; Kelley and Jack, 2002).
Leaf litter nutrient content influences mass loss in temporary wetlands and adjacent uplands (Day, 1982; Palik and Batzer, 2006). Leaf litter mass losses were more rapid for litters with high initial phosphorus or low C/N ratio (Day, 1982; Palik and Batzer, 2006). Leaf litter mass loss rates were lower and followed a negative exponential pattern (Day, 1982; Shure et al., 1986; Battle and Golladay, 2001; Palik and Batzer, 2006) in litters with high C/N ratio, low phosphorus, or physiochemical barriers to decomposition such as lignin or waxy cutin. Within Minnesota vernal pools mass loss rate increases due to inundation were similar for sugar maple, trembling aspen (*Populus tremuloides*), and black ash (*Fraxinus nigra*) (Palik and Batzer, 2006). This suggests that the effect of inundation does not depend on litter species.

1.4 Problem Formulation

Vernal pools are considered to be ‘hot spots’ of carbon cycling within the forested landscape. A key component of the carbon cycle within vernal pools is the decomposition of organic matter, largely leaf litter from surrounding overstory. The cyclical hydrology typical of vernal pools leads to moisture and oxygen conditions which favor leaf litter decomposition (Barlocher et al., 1978; McClain et al., 2003; Hunter, 2008). Periodic dry-down events within vernal pools may increase oxygen availability in decomposing litter, therefore maintain higher microbial activity, compared to permanent flooding (Battle and Golladay, 2001; Langhans and Tockner, 2006; Inkley and Wissinger, 2008). Compared to non-flooding upland, the activity of macroinvertebrates can be high within vernal pools due to high moisture conditions (Battle and Golladay, 2001; Batzer et al., 2004). The presence of surface water in vernal pools could also lead to rapid leaching loss from litter. The effect of cyclical hydrology on decomposition is
highly dependent on the hydroregime of individual pools. In order to evaluate if vernal pools are 'hot spots' of decomposition, investigations must include not just the differences in decomposition rates over time between pool and upland, but also the biological and physical mechanisms underlying this difference.

This thesis addresses the claim that vernal pools represent a 'hot spot' of decomposition. This knowledge is needed to assess the importance of vernal pools to carbon cycles within the forested landscape of central Ontario, and how climate change may alter that relationship (McClain et al., 2003; De Meester et al., 2005). If vernal pools within the present study area are decomposition 'hot spots', then I hypothesize that the amount and rate of litter mass loss will be greater in vernal pools than adjacent upland. To determine why decomposition may differ between pool and upland, potential mechanisms driving those differences were explored.

If the differences in mass loss are driven by biological mechanisms, then the impact of decomposer organisms on mass loss will differ between pool and upland. Shifts in macroinvertebrate, fungi, and bacterial decomposer communities within litter may influence both the litter mass loss, and the chemical processes within the litter. The impact of specific communities on decomposition can be assessed using fine and coarse mesh bags, which sort organisms roughly by size. Changes in chemical processes in litter are often tracked through changes of carbon, nitrogen, and carbon to nitrogen ratio, which can be indicative of differences in litter quality and decomposer activity.

If the differences in mass loss are driven by physical mechanisms within the vernal pools, then differences in the physical pool environment will alter the amount and rate of mass loss. The properties of the water in the pools, such as temperature and solute
concentration, may influence early mass loss due to leaching. The physical confinement of the litter within litterbags may also affect mass loss in both early and mid-stage decomposition. The hydrology and water chemistry of pools over time may influence mass loss rates in vernal pools.

Given the high variation in hydrology, geology, and community composition between vernal pools, a high degree of replication is needed to accurately characterize decomposition rates. Most studies of decomposition within temporary wetlands have been conducted with very limited spatial (<4 pools) and temporal (1 year) replication (Lockaby et al., 1996; Ellis et al., 1999; Battle and Golladay, 2001; Kelley and Jack, 2002). In this study this variability was addressed by utilizing a novel degree of spatial (24 pools) and temporal (2 years) replication.
CHAPTER TWO
METHODS

2.1 Study Area

The field study was conducted at the Frost Centre Area in Central Ontario (Figure 2). The geographical centre of the study area was located at 45.1587 North, 78.8460 West (UTM 17N Easting 669298.7 Northing 5002837.2). The Frost Centre Area consists of approximately 24,000 hectares of provincially owned mixed-wood forest within the Great Lakes-St. Lawrence forest region (Frost Centre Working Committee, 2001). The landscape within the study area consists of gently rolling uplands alternating with lowlands containing numerous lakes, rivers and wetlands. The upland forest is dominated by red and sugar maple (Acer rubrum L., A. saccharum Marsh.) and pine spp. (Pinus strobus L., P. resinosa Ait.) (Burns and Honkala, 1990). The Frost Centre Area is managed by the Ontario Ministry of Natural Resources for mixed use, including timber harvesting, scientific research, and recreational activities (Frost Centre Working Committee, 2001).
**Figure 2.** Overview of the study area

The study area of field experiment, including survey area boundaries ( ), location of identified vernal pools (●), lakes and wetlands. Vernal pools locations were identified in a field survey of 2.8 km² within the Frost Centre Area. The study was conducted within a mixed-wood upland forest in Central Ontario, Canada. Areas of high (■), low (■), and minimal (■) vernal pool density were determined from field-identified vernal pool locations.
2.2 *Vernal pool characterization*

2.2.1 Vernal pool location and identification

A terrestrial field survey was conducted between May and August 2008 focusing on areas ≤ 500 m from established roads or trails likely to contain vernal pools (Figure 2). Vernal pools are more likely to occur in areas with a slope less than 10° that contain small, isolated depressions (Creed et al., 2003; Grant, 2005). Potential vernal pools were identified where standing or seeping water was present without any identifiable overland hydrological connection (Figure 3). No minimum size requirement was imposed on identified pools, which differed from previous studies (Palik et al., 2003; Colburn, 2004; Grant, 2005). Vernal pools identified followed the definition of Colburn (2005). Each pool was found within a woodland context, was less than 1 ha in surface area, and had cyclical hydrology that shifted annually between standing water and dry basin. This excluded both permanently flooded wetlands and temporary streams. Colburn's definition of vernal pools was used to ensure that the unique hydrology of vernal pools was present within identified vernal pools.

The spatial coordinates of each vernal pool was determined using an eTrexH handheld GPS (Garmin, Olathe, KS), with an average horizontal accuracy of ± 15m. All initial pool characteristics were observed at time of identification. Water depth (m) was measured at the point of maximum depth. Maximum length and width of the pool basin was measured using a Leica DISTO D2 laser distance meter (Leica Geosystems AG, Heerbrug, Switzerland). The margin of each pool basin was visually identified by vegetation changes as well as the presence of matted leaves and algae (Wilcox and Los Huertos, 2005). Plant species present in both the understory and overstory of each pool
were visually identified and recorded. Each vernal pool was re-visited during a second survey of all sites between August and October 2008. Sites at which standing water was still present were categorized as permanent wetlands and excluded from further analysis.

2.2.2 Vernal pool selection

Twenty-five vernal pools were selected for detailed study based on pool basin area and canopy species. Selected pools each had a maximum basin area greater than 5.0 m². Basin area was approximated by assuming an elliptical pool shape and utilizing the length and width as axes (Millar, 1971; Brooks and Hayashi, 2002; Batzer et al., 2004). Pools were reasonably free of shrubby growth to allow access and deployment of litterbags. All selected pools were located in stands dominated by red maple alone, or red maple and Eastern hemlock (*Tsuga canadensis* (L.) Carriere). One pool was subsequently identified as an ephemeral stream, and was excluded from the field experiment. All analyses were conducted on the remaining twenty-four vernal pools.
**Figure 3.** Example of a Central Ontario vernal pool through the seasons

A vernal pool within the study area at four time points from October 2008 to September 2009. Vernal pools within the study area tended to fill partially in late fall. Pools became fully inundated in spring. Most pools dried completely by late summer or early autumn. Pools which did not dry completely in 2008 were categorized as permanent wetlands and excluded from further analysis.
2.2.3 Vernal pool characterization measurements

The 24 selected vernal pools were visited approximately bi-weekly during the snow-free period from May 11th 2009 to October 31st, 2009. A total of 11 visits were made to each pool in this time period. During the first visit of 2009 a bamboo pole was placed at the location of maximum water depth to establish a fixed reference point for subsequent measurements. Transects along the N-S and E-W cardinal axes were established, centered on the reference pole. During each visit water depth was measured at the central reference pole. The extent of standing water determined along the two cardinal transects using a Leica DISTO D2 laser distance meter. At each visit standing water was present, the water temperature (°C), pH and dissolved oxygen (mg/l) were measured within 5 meters of the central pole and at least 2cm above the pool bottom using a YSI 600 QS Multiparameter Water Quality Sonde (YSI Environmental Monitoring Systems Incorporated, Yellow Springs, OH).

The hydrological regime of each pool was represented by hydroperiod, defined as the number of days during which water was present within each pool between October 31st, 2008 and October 31st, 2009. The water capacity of each pool was represented by the maximum water depth and maximum surface area, measured at the time of maximum flooding extent for each pool. The time at which maximum water extent occurred in 2009 was not the same for all pools, but tended to occur either in May or October. Maximum water surface area was approximated by assuming an elliptical pool shape and utilizing the maximum N/S and E/W extent as axes (Millar, 1971; Brooks and Hayashi, 2002; Batzer et al., 2004). Raw field measurements of water temperature (°C), pH, and dissolved oxygen (mg/l) were used in analyses.
2.3 Experimental Design

This study was designed to test the differences in leaf litter decomposition rates between vernal pool and upland habitats. The litterbag method was used to compare the rate and amount of litter mass loss between vernal pool and upland habitats a 24-month period. Twenty-four sites were chosen within the study area to capture potential spatial variability among vernal pools. Each site contained both pool and upland habitat in order to minimize variation due to environmental differences. To observe mass loss in two distinct years, litterbags were deployed at both habitats of each site in October of 2008 and 2009. Coarse mesh (3mm) litterbags were used to ensure that large shredding macroinvertebrates have access to the enclosed litter. Litterbags were recovered in sets of four at both habitats of each site repeatedly over the course of 24 and 12 months for litterbags deployed in 2008 and 2009, respectively.

This study represents a fully-crossed design with replication across sites and repeated measures over time for each habitat type at each site. Each site represented one replication, containing both pool and upland habitat treatments. Each habitat type within each site represented one experimental unit. Each set of four litterbags represented the sampling unit from a specified habitat and site. Each individual litterbag represents a sub-sample at a specified habitat, site, and time. The rate and amount of mass loss was compared between pool and upland across sites, at 6, 12 and 24 months.

The difference in mass loss due to different sizes of decomposer organisms after 12 months of decomposition was compared using the litterbag method with two different mesh sizes. Coarse mesh litterbags were used as described above. Fine mesh litterbags (0.5mm) were used to exclude macroinvertebrates from the decomposition of contained
litter (Kampichler and Bruckner, 2009). Fine mesh litterbags were removed after 12 months of decomposition for both deployment years. This represents a fully-crossed statistical design, with year, habitat, and mesh size treatments.

The content of carbon (C), nitrogen (N), and the carbon to nitrogen ratio (C:N) in litter from coarse mesh litterbags was compared between pool and upland habitats after 12 months of decomposition. This represents a fully-crossed statistical design, with time and habitat treatments. Mass loss rate from coarse mesh litterbags in vernal pools after 12 months of decomposition was compared to vernal pool characteristics. Characteristics were measured at each pool bi-weekly from May to October 2009. Characteristics used reflected those which may influence decomposition rates: hydroperiod (days inundated), dry-down events (number of times pool dried out between May and October), pH, dissolved oxygen, and temperature of the water.

The amount and rate of mass loss due to leaching was tested using microcosms in order to allow manipulation of the physical environment. Parameters of the microcosms were chosen in an attempt to replicate natural conditions under investigation, specifically leaching. Environmental conditions manipulated were chosen to reflect those that may affect amount or rate of leaching loss. Each manipulation of an environmental condition was considered a separate experiment.

Due to high spatial and temporal variation in the system under investigation, including hydrology, community, and ecosystem processes, error control was an important aspect of the experimental design of this study. To reduce the variation in litter chemistry, a single species, red maple, was used for all mass loss observations. Red maple was the dominant or co-dominant canopy species at all sites, and therefore naturally present in
the selected pools. The mass of the litterbags themselves may have changed over time due to leaching or growth of organic material on the bag. To account for this change one of the four litterbags removed from each habitat and site at each recovery time was left empty. To address variation in mass loss between litterbags from the same habitat at the same site, three of the four litterbags removed from each habitat and site at each recovery time were filled with litter. To measure mass loss due to handling during deployment and recovery, a set of litterbags (3 filled, 1 empty) were recovered immediately after litterbag deployment in 2008.

### 2.4 Field experiment

#### 2.4.1 Litterbag construction

Litterbags used in this study consisted of 22 cm x 25 cm polyester mesh bags filled with 10 (± 0.05) g air-dried red maple leaf litter. Two polyester mesh fabrics were used: a coarse mesh with 3mm square openings (Java Collection Sheer, Item No. 979100, Fabricland, Toronto, ON) and a fine mesh with 0.5mm square openings (Drapery Sheers, UPC 624820511212, Wal-Mart Canada, Missisauga, ON). All litterbags were sewn together with polyester upholstery thread (Gütermann creativ Extra Strong M 782, Gutach-Breisgau, Germany). Polyester was chosen as the primary construction material as it had been used in previous litterbag studies (Gholz et al., 2000) and is highly resistant to degradation due to leaching, microbial action, and exposure to UV. Nylon cord (CRD-02, Canadian Drapery Hardware, Toronto, ON) was sewn into the litterbags to allow attachment to wooden stakes during field deployment. The mass of each unfilled litterbag was measured using an electronic balance to a precision ± 0.01g.
Leaf litter used in this experiment was recently fallen red maple leaves collected by hand from the forest floor.

Litter collection took place in the general study area during September and October prior to deployment each year. The litter was air-dried indoors for at least 5 days at room temperature. To determine the ratio of oven-dry to air-dry litter mass a 10.00 (± 0.05) g sample of litter was taken for every 75 litterbags filled. Litter samples were oven-dried at 40°C until a constant mass was reached. The final oven-dry mass of each sample was recorded. The ratio of oven-dry to air dry mass was calculated for each sample, and averaged across all samples within each deployment year. The oven-dry mass of litter placed within each litterbag was calculated by multiplying the recorded air-dry litter mass for each litterbag by the average ratio of oven-dry to air-dry litter mass.

Three of every four litterbags were filled with 10.00 (± 0.05) g litter each. The remaining bags were deployed without litter in order to measure mass accumulation or loss in the absence of litter. The open side of all litterbags were sewn closed. The mass of the empty litterbags was measured to determine the average mass of thread used to close each bag. The mass of each filled litter bag was calculated as the sum of the mass of the litterbag, mass of litter, and the mass of thread used for final closure.

2.4.2 Litterbag deployment

Litterbags were deployed at twenty-four sites selected as per above. All bags were deployed between Oct 15th and 31st of each year. At each site a total of 48 coarse mesh bags were deployed in 2008 and 24 coarse mesh bags in 2009. Eight fine mesh bags were deployed at each site in both 2008 and 2009. An equal number of bags were placed in the pool and upland habitats of each site.
Vernal pool litterbags were placed within the pool basin, between the point of maximum depth and the maximum flooding extent (Figure 4a). Upland litterbags were placed on non-flooding upland no greater than 10 m from the pool litterbags (Figure 4a). Both pool and upland litterbags were placed on areas of relatively flat, open ground with minimal understory vegetation and woody debris.

Litterbags were arranged into sets of four bags, three filled and one empty. Each set was tied to a nylon cord, with the position of the empty litterbag on each cord randomly determined (Figure 4b). Litterbag sets were secured to the forest floor between a wooden stake and metal garden stake (Figure 4b). Litterbags were arranged in a fan-shaped pattern originating at the wooden stake. Each litterbag was at least 20cm away from any adjacent litterbag (Figure 4b).
Within each site (a), litterbags were deployed both within pool and upland habitats, within 10 m of each other. At each habitat (b), litterbags were grouped into four-bag litterbag sets, then secured to a wooden stake with a nylon cord. Each litterbag set consisted of three filled and one empty litterbag.
2.4.3 Litterbag recovery

For each recovery date a set of four litterbags, three filled and one empty, was removed from each pool and upland location. Each litterbag set removed was randomly selected from within each habitat and site combination. The coarse mesh litterbags deployed in 2008 were recovered after 0, 6, 8, 10, 12 and 24 months of field incubation. The coarse mesh litterbags from the 0 month recovery date were collected immediately after deployment in order to determine mass loss due to handling. Coarse bags deployed in 2009 were recovered after 6, 8 and 12 months. Fine mesh litterbags deployed in 2008 and 2009 were recovered after 12 months.

After recovery, vegetation growing from the surface of the bag, including green shoots and fungal fruiting bodies, was removed by hand. Litterbags were first air dried for up to 5 days, then dried at 40°C until a constant mass was reached. Moss, algae and other residues on the surface of the bag were removed following drying. The oven-dry mass of each bag was determined to a precision of 0.01g with an electronic balance.

2.4.4 Calculation of mass loss

Litter mass loss was calculated for each litterbag recovered. The litter mass loss of each filled litterbag was determined by subtracting litter mass at recovery from initial litter mass. The mass loss of each non-filled litterbag was calculated by subtracting the litterbag mass at recovery from initial litterbag mass.

Three empirical estimates of experimental error were used to adjust the raw litter mass loss values. To adjust for moisture remaining in litter at time of deployment, the initial air-dried litter mass was multiplied by the ratio of oven-dry to air-dry litter mass prior to calculation of litter mass loss. The ratio of oven-dry to air-dry litter mass was 0.916.
(±0.0039) in 2008, and 0.932 (± 0.0030) in 2009. To account for mass loss due to handling, the litter mass loss at the 0 month recovery time was subtracted from initial litter mass of all litterbags at the same pool. The average litter mass loss from coarse litterbags at 0 month recovery was 0.113 (±0.0131) g, approximately 1% of the initial mass. Litter mass loss due to handling from fine mesh litterbags was assumed to be zero because fragmented litter was observed to be held within the fine mesh litterbags. To account for mass change of the litterbag itself, the mass lost from non-filled litterbags was subtracted from the mass lost from each filled litterbag in the same four-bag recovery set of litterbags. The average mass lost from non-filled bags was - 0.35 (± 0.022) g. The adjusted litter mass loss was expressed as a percent of the initial oven-dry litter mass.

2.4.5 Determination of carbon, nitrogen and ash content

The carbon, nitrogen, and ash content of leaf litter was determined for litter samples taken from litterbags deployed in October 2008 and recovered after 0 and 12 months. Litter samples were ground with a Wiley Mill using a mesh size of 20 mm. Ground litter samples were oven dried at 40°C until a constant mass was reached.

The content of C and N in recovered leaf litter was determined by combustion of sub-samples from each litterbag. All nutrient contents are reported as percent of oven dry litter mass. Total carbon content of litter was analyzed on 10 (± 1) mg sub-samples using a LECO CR-12 Carbon Determinator (LECO Corporation, St Joseph, MI) calibrated with orchard leaves (C = 44.97 (± 0.49) %, LECO Corporation, St Joseph, MI). Total nitrogen content of litter was analyzed on 5.0 (± 0.1) mg sub-samples using a LECO FP-428 Nitrogen Protein Analyzer (LECO Corporation, St Joseph, MI) calibrated with EDTA
(N=9.57 (± 0.02) %, LECO Corporation, St Joseph, MI). Ash content of litter was analyzed on 1.00 (± 0.005) g sub-samples using a muffle furnace set at 575°C for 24 hours.

Carbon and nitrogen content as reported for each litterbag was adjusted for ash content, in order to correct for the inorganic material in litter. The mass of each nutrient within each litterbag was calculated by multiplying the percent nutrient content by oven-dry litter mass. The ash-free dry mass (AFDM) of leaf litter within each bag was then calculated by multiplying the ash content by the oven-dry litter mass. The mass carbon and nitrogen content within each litterbag were then divided by ash-free dry mass, to generate carbon and nitrogen content. The carbon to nitrogen ratio (C:N) of each litter sample was determined by dividing carbon by nitrogen content for each litterbag. The carbon, nitrogen, and C:N values were averaged across each set, and used in all subsequent analyses.

2.5 Statistical analysis

2.5.1 Time course of mass loss

Leaf litter mass loss from coarse mesh litterbags was compared between deployment years (2008, 2009) and habitats (pool, upland) at each recovery time. To determine if loss was significantly different between year or habitat a fully-crossed mixed model ANOVA analysis was conducted (SAS 9.2, Proc Mixed). Habitat was included in the analysis as a fixed effect, while site and year as random effects (Bowley, 2008). Sampling over time from the same site was treated as repeated measures over recovery time (t, days), using the AR1 covariance structure. This structure assumes that data points measured closer
together in time are more highly correlated, as is the case in repeated measure experiments (Kowalchuk et al., 2004).

Leaf litter mass loss rate (k, % day⁻¹) was determined for pool and upland in both deployment years. Mass loss over time was assumed to follow a exponential model. This model is widely used to calculate and compare decomposition rates (Olson, 1963; Graca et al., 2007; Kampichler and Bruckner, 2009). Mass loss over time (t) for each habitat (pool, upland) and year (2008, 2009) combination was fit separately to a negative exponential model across the 24 pools at which litter mass loss was observed (SAS 9.2, Proc NLIn). The litter mass loss at t=0 was assumed to be 0% for all analyses. The exponential decay constant calculated from each fitted model is assumed to approximate daily mass loss rate (Graca et al., 2007).

Two regression analyses were conducted for 2008 litterbags. The first included data from 0 to 24 months, using data from 6 recovery dates. The second included data only from 0 to 12 months, using data from 5 recovery dates. The latter allowed for comparison of k values between deployment years. For 2009 litterbags one regression analysis was conducted using data from 0 to 12 months, using data from 3 recovery dates. First-year decay constants were compared between habitats and deployment years to determine if mass loss rates differed significantly between year or habitat. A fully-crossed mixed model was used (SAS 9.2, Proc Mixed). Habitat was analyzed as a fixed effect, while site and year as random effects (Bowley, 2008).

2.5.2 Effects of vernal pool characteristics on mass loss rate

The relationship between litter mass loss rate (k) and vernal pool characteristics was tested. Mass loss rates were calculated from litterbags deployed in October 2008, and
recovered in October 2009 and 2010 for 12 and 24 months of decomposition, respectively. Vernal pool characteristics of hydroperiod (days inundated) and dry-down events (no. times pool basin dried out) between October 2008 and October 2009, as well as water temperature, pH, and dissolved oxygen measured in October 2009 were tested. Vernal pool characteristics were not monitored in 2010, therefore measurements taken in the 2008-2009 period were used in the analysis of both 12 and 24 month mass loss rates. A forwards stepwise linear regression was used to select the pool characteristics which best predicted mass loss rate (SAS 9.2, Proc Reg, entry=0.15, stay=0.10). Pool characteristics were added to the model one at a time if the significance level of their F-statistic was below 0.15. Pool characteristics were retained within the model if the significance level of their F-statistic remained below 0.10 with the inclusion of additional variables (Bowley, 2008).

2.5.3 Effect of litterbag mesh size on mass loss
The effect of mesh size (coarse = 3 mm, fine = 0.5 mm) and habitat (pool, upland) on the litter mass loss after 12 months of decomposition was evaluated for both deployment years. A fully-crossed mixed model analysis was used (SAS 9.2, ProcMixed). Mesh size was treated as a fixed effect. Site and year were treated as random effects. Post-hoc comparisons of the average litter mass loss were made for each combination of habitat and mesh size (SAS 9.2, Proc Mixed, t-test with Tukey’s Adjustment).

2.5.4 Changes in carbon, nitrogen and ash content of litter
The carbon, nitrogen and ash content, as a percent of ash-free dry mass, as well as the carbon to nitrogen ratio, were compared between decomposition times (0 month, 12 months) and habitats (pool, upland). A mixed model analysis was used. Decomposition
time and habitat were treated as fixed effects and site as a random effect (SAS 9.2, Proc Mixed).

2.6 Laboratory leaching experiments

Four laboratory experiments were conducted to further investigate early mass loss due to leaching within vernal pools. A common microcosm design was used for all experiments to mimic the environment of leaf litter in vernal pools at the time of fall re-filling. The rate of mass loss over time due to leaching was investigated up to 42 days. The influence of water temperature, leachate concentration in water, and litter enclosure method were also tested.

2.6.1 Experiment 1: Time course of mass loss from unconfined litter

2.6.1.1 Microcosm construction

Each microcosm was constructed within a lidded, opaque plastic container measuring 22x22x43cm with a volume of 21 L. 33.68 (± 0.05) g of air-dried red maple leaf litter was placed in each microcosm. Mass of litter in each microcosm replicated the average density (349.3 g m⁻²) of litter intercepted between September and November 2008 in 66 litter traps placed within the Frost Centre Area. Litter was collected and air-dried as described above for field experiment (See Section 2.4.1). Litter was placed unconfined in each microcosm to emulate natural conditions. Ten 33.68 (± 0.05) g samples of litter were taken per experiment to determine the ratio air-dry to oven-dry litter, and treated as described above for field experiment (See Section 2.4.1).

Untreated well water (19 L) was added to each microcosm to emulate standing water within vernal pools. Untreated well water was used to emulate precipitation and
groundwater accumulating in natural pools. The water depth within microcosms, approximately 20 cm, corresponds to the mean water depth in 24 experimental pools measured in October 2009.

The microcosms were incubated at 5.0 (± 3.0) °C within an Environmental Chamber (Model No. C905, Conviron Controlled Environments, Winnipeg, MB). This approximates the average late October water temperature, 6.37(± 0.24) °C, measured in this study (Figure 5). This temperature also minimizes biotic activity that may contribute to mass loss, allowing the effects of leaching to be isolated.

2.6.1.2 Microcosm initiation and recovery

All microcosms were initiation on the same day, and placed randomly within the environmental chamber. Five microcosms were recovered from the environmental chamber after 0, 3.5, 7, 14, and 28 days. Microcosms were recovered randomly at each recovery time. A recovery time of 0 indicates that the microcosm was prepared as indicated then removed immediately from the environmental chamber.

At the time of recovery loose litter was separated from water in microcosm via filtering apparatus. Each filter apparatus consisted of a J Cloth (Associated Brands, Missisauga, ON) pre-weighed to a precision of 0.01g placed on top of a metal colander. Litter was air dried for up 5 days, then oven dried at 40°C for at least 4 days, until a constant mass was reached. Oven-dry mass of litterbags and loose litter with filter cloth were measured to a precision of 0.01g.
2.6.1.3 *Calculation of mass loss*

The litter mass loss was calculated for each microcosm. The litter mass loss for each microcosm was determined by subtracting litter mass at recovery from initial litter mass. To adjust for moisture remaining in litter at time of deployment, the initial air-dried litter mass was multiplied by the ratio of oven-dry to air-dry litter mass prior to calculation of litter mass loss. The ratio of oven-dry to air-dry litter mass was 0.967.

2.6.1.4 *Statistical Analysis*

The rate of dry mass loss over time was characterized by fitting an exponential function over a 28 day period (SAS 9.2, Proc NLin). The exponential decay constant was assumed to approximate the daily rate of mass loss (k, % day⁻¹). The litter mass loss at t = 0 was assumed to be 0%.

2.6.2 *Experiment 2: Time course of mass loss from confined and unconfined litter*

2.6.2.1 *Microcosm construction*

Microcosms were constructed as described for Experiment 1, except where noted below (See Section 2.5.1.1).

Each microcosm contained a total of 33.68 (± 0.05) g air-dried red maple leaf litter, either confined in a litterbag or unconfined within the microcosm. 10.0 (± 0.05) g of litter was placed with a coarse mesh (3mm) litterbag, prepared as above in field experiment (See Section 2.4.1). The remaining 23.68 (± 0.05) g of litter was placed unconfined within the microcosm as in Experiment 1.
2.6.2.2 *Microcosm deployment and recovery*

Microcosms were deployed and recovered as described for Experiment 1, except where noted below (See Section 2.5.1.2).

Litter was recovered from microcosms after 0, 3.5, 7, 14, 28, and 42 days. Three microcosms were recovered at each recovery time. Recovered litterbags were treated as described for the field experiment (See Section 2.4.3).

2.6.2.3 *Calculation of mass loss*

Litter mass loss was calculated as described for Experiment 1, except where noted below (See Section 2.5.1.3).

The ratio of oven-dry to air-dry litter mass was 0.976. It was assumed that the mass of the litterbag was not affected by microcosm.

2.6.2.4 *Statistical Analysis*

Litter mass loss rate was calculated as described for Experiment 1, except where noted below (See Section 2.5.1.4). The negative exponential function was fit over a 42 day period (SAS 9.2, Proc NLin).

The litter mass loss was compared across removal times and litter confinement using a fully-crossed mixed model analysis (SAS 9.2, Proc Mixed). Removal time and confinement type were treated as a fixed effect (Bowley, 2008). Post-hoc comparisons of the litter mass loss were conducted for each combination of litter confinement and removal time (SAS 9.2, Proc Mixed, t-test with Tukey’s Adjustment).
2.6.3 Experiment 3: Effect of water temperature on litter mass loss

2.6.3.1 Microcosm construction

Microcosms were constructed as described for Experiment 1, except where noted below (See Section 2.5.1.1).

Ten microcosms were incubated at a temperature of 5.0 (± 3.0) °C and ten at room temperature, approximately 20 °C.

2.6.3.2 Microcosm deployment and recovery

Microcosms were deployed and recovered as described for Experiment 1, except where noted below (See Section 2.5.1.2).

Litter was recovered from all microcosms after 28 days.

2.6.3.3 Calculation of mass loss

Litter mass loss was calculated as described for Experiment 1, except where noted below (See Section 2.5.1.3).

The ratio of oven-dry to air-dry litter mass was 0.978 (±0.0039).

2.6.3.4 Statistical Analysis

Litter mass loss after 28 days was compared between treatments (5.0°C, 20.0°C). A mixed model analysis was used (SAS 9.2, ProcMixed), with treatment of water temperature treated as fixed effects.
2.6.4  Experiment 4: Effect of leachate concentration and litter confinement on litter mass loss

2.6.4.1  Microcosm construction

Microcosms were constructed as described for Experiment 1, except where noted below (See Section 2.5.1.1).

Two litter confinement treatments (litterbag, unconfined) and three leachate concentrations (0%, 50%, 100%) were used, fully crossed. Litterbag confinement treatments were prepared as described for Experiment 2 (See Section 2.5.2.1). Leachate consisted of the water recovered from the 5°C treatment of Experiment 4 upon its completion (See Section 2.5.3.1). The leachate was added to microcosms at a rate of 0%, 50%, and 100% of the total liquid, with the remainder being untreated well-water as above.

2.6.4.2  Microcosm deployment and recovery

Microcosms were deployed and recovered as described for Experiment 1, except where noted below (See Section 2.5.1.2).

Litter was recovered from all microcosms after 28 days.

2.6.4.3  Calculation of mass loss

Litter mass loss was calculated as described for Experiment 1, except where noted below (See Section 2.5.1.3).

The ratio of oven-dry to air-dry litter mass was 0.929.
2.6.4.4 Statistical Analysis

Litter mass loss after 28 days was compared between treatments of leachate concentration (0%, 50%, 100%) and litter confinement (litterbag, unconfined) using a mixed model ANOVA analysis (SAS 9.2, ProcMixed). Treatments were treated as fixed effects. Post-hoc comparisons of the litter mass loss were made for each combination of litter confinement type and leachate concentration (SAS 9.2, Proc Mixed, t-test with Tukey’s Adjustment).
CHAPTER THREE

RESULTS

3.1 Vernal pool characteristics

The 88 vernal pools identified within the study area occurred on slopes ranging from 0° to 6.8°, with a mean slope of 2.8°. Vernal pool density within the surveyed area was 31.4 pools km\(^{-2}\). The estimated pool density within the Frost Centre Area was 24.4 pools km\(^{-2}\) (Appendix 3).

Of the 24 vernal pools monitored in detail, all contained standing water in late October 2008. Eighty percent of these pools dried completely at least once between June and September 2009. Vernal pool hydroperiod was expressed as number of days standing water was present between October 31st 2008 and October 31st 2009. Hydroperiod ranged from 218 days to 365 days, with an average (± SE) of 303 (± 8.65) days (Table 10). This indicates that most pools in this study dried in July, and remained dry for less than 10 weeks.

The maximum water depth recorded at each vernal pool ranged from 7 cm to 50 cm, with a mean (± SE) of 23 (± 2) cm (Table 10). The maximum depth occurred in October for 64% of pools. Maximum depth in the remaining pools occurred in late May or early June. Water depth declined rapidly in late June, and water levels stayed below spring and fall values throughout July, August, and September (Figure 5a). The water depth increased episodically in mid-July and Mid-August as a result of local precipitation. The maximum surface area calculated for each pool ranged from 3.8 m\(^2\) to 529.3 m\(^2\), with a mean (±SE) of 68.2 (± 12.08) m\(^2\).
Water temperatures recorded ranged from 1.14 °C to 21.1 °C, with a mean of 12.4 (± 0.32) °C across all pools and time points when water was present (Figure 5b). The spring minimum water temperature was 10.2 (± 0.51) °C in late May. Water temperatures increased from June until August, with peak temperatures of 16.9 (± 0.56) °C in mid-June and 19.5 (± 0.56) °C in late August. Temperatures dropped steadily from August to October. The lowest water temperature recorded, 6.37 (± 0.24) °C, occurred in late October.

The water pH recorded ranged from 3.32 to 5.42, with a mean of 4.30 (± 0.03) across all pools and time points when water was present (Figure 5c). The average pH across all pools was 3.92 (± 0.124) in early May, increased to a maximum of 4.65 (± 0.081) in early June. The average pH declined to the minimum, 3.90 (± 0.111), in late August, then increased from August to October.

Dissolved oxygen content (mg/l) recorded ranged from 0.58 mg/L to 9.10 mg/l with a mean of 3.17 (± 0.13) mg/l across all pools and time points when water was present (Figure 5d). The average dissolved oxygen content across all pools was greatest in early May (4.23 (± 0.46) mg/L). Average dissolved oxygen content declined rapidly through May and June. The minimum average dissolved oxygen content, 1.364 (± 0.148) mg/L, was reached in mid-June. Dissolved oxygen content then fluctuated near the mean oxygen content from June to October.
**Figure 5.** Vernal pool characterization summary

The average (± SE) water (a) depth (% maximum), (b) temperature (°C), (c) pH, and (d) dissolved oxygen (mg/l) measured bi-weekly at 24 vernal pools between May 11th 2009 to October 31st, 2009. Vernal pools were located in a central Ontario mixed-wood forest. Depth was measured at the point of maximum depth within each pool. All other measurements were taken within 5 metres of the central pole.
3.2  Field experiment

3.2.1 Time course of mass loss

The litter mass loss from coarse mesh (3mm) litterbags over 24 months of decomposition followed a negative exponential pattern in both vernal pool and adjacent upland habitats (p<0.0001; Figure 6, Table 1). The first set of litterbags was deployed in October 2008 and collected over the course of 24 months (October 2008 to October 2010). Litter mass loss after 6 months of decomposition was 38.5 (± 1.0) % and 34.0 (± 0.8) % in pool and upland habitats, respectively. After 12 months of decomposition, mass loss was 45.2 (±0.9) % in pool and 45.1 (±0.7) % in upland. After 24 months of decomposition, mass loss was 52.55 (± 1.14) % in pool and 55.2 (± 1.3) % in upland. The second set of litterbags was deployed in October 2009 and collected over the course of 12 months (October 2009 to October 2010). Litter mass loss from the pool habitat was 35.1 (± 1.0) % and 43.0 (± 0.9) % after 6 and 12 months of decomposition, respectively. Litter mass loss from the upland habitat was 27.4 (± 0.7) % and 39.7 (± 0.9) % after 6 and 12 months of decomposition, respectively.

Mass loss from the first litterbag deployment was significantly greater in the pool habitat than upland after 6, 8 and 10 months of decomposition (p<0.05; Figure 6a, Table 2a). Mass loss after 24 months of decomposition was significantly lower in the pool habitat than upland (p< 0.05; Figure 6a). In the second litterbag deployment, mass loss was significantly greater in the pool habitat after 6, 8, and 12 months of decomposition (p<0.05; Figure 6b, Table 2b).

The litter mass loss rate (k, % day⁻¹) after 12 months of decomposition in the pool habitat was 2.09 x 10⁻³ % d⁻¹ and 1.82 x 10⁻³ % d⁻¹ for first and second deployments,
respectively. The loss rate in the surrounding upland was $1.88 \times 10^{-3} \% \, \text{d}^{-1}$ and $1.46 \times 10^{-3} \% \, \text{d}^{-1}$ in first and second deployments, respectively. The loss rate differed significantly between pool and upland for both deployment years ($p<0.001$). The loss rate after 24 months of decomposition was $1.68 \times 10^{-3} \% \, \text{d}^{-1}$ and $1.59 \times 10^{-3} \% \, \text{d}^{-1}$ in pools and upland respectively. Loss rate differed significantly between habitat types after 24 months of decomposition ($p<0.01$).
Figure 6. Time course of litter mass loss

The litter mass loss from coarse mesh (3mm) litterbags over (a) 24 months and (b) 12 months of decomposition at pool and upland habitats. Litter mass loss was significantly explained by a negative exponential model ($R^2=0.97 – 0.99$, $p<0.0001$). Pair wise comparison between litter mass loss from each habitat was conducted at each removal time, with significantly different pairs marked by (*). Mass loss was expressed as a percent of the initial oven-dry litter mass. Mass loss was measured in both pool and upland habitats at 24 sites in a central Ontario mixed-wood forest.
Table 1. Mean litter mass loss across time, habitat, and mesh size

Mean leaf litter mass loss from coarse mesh (3 mm) and fine mesh (0.5 mm) litterbags over up to 24 months of decomposition at pool and upland habitats. Mass loss was expressed as a percent of the initial oven-dry litter mass. Mass loss was measured in both pool and upland habitats at 24 sites in a central Ontario mixed-wood forest.

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Table 2. Comparison of mass loss across habitat, site, and time

Comparison of litter mass loss across habitat type, site and time for coarse-mesh (3mm) litterbags deployed in October (a) 2008 and (b) 2009. Mass loss was measured at 24 sites each containing two habitat types (pool, upland). For 2008 deployment there were 6 recovery times over 24 months. For 2009 deployment there were 3 recovery times over 12 months. Comparison was conducted for each year with a fully-crossed mixed model ANOVA (SAS 9.2, Proc Mixed) utilizing repeated measures over recovery time. Habitat was analyzed as a fixed effect, while site and time as random effects (Bowley, 2008).

(a)

Covariance Parameter Estimates

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### Fixed Effects

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3.2.2 Effect of vernal pool characteristics on mass loss rate

The litter mass loss rate (k) within vernal pools after 12 months of decomposition was significantly explained by hydroperiod (days flooded) and October 2009 water temperature (°C) (p<0.01, Figure 7, Table 3). The loss rate was greater in pools with longer hydroperiods and lower water temperatures. The model explained 30.2% of variation in 12-month loss rates between pools, with 21.9% explained by temperature alone and 19.4% explained by hydroperiod alone. The loss rate within vernal pools after 24 months of decomposition was significantly explained by hydroperiod (p<0.05). The loss rate was greater in pools with longer hydroperiods. The model explained 27.0% of variation in 24-month loss rates between pools.

3.2.3 Effect of litterbag mesh size on mass loss

The litter mass loss from fine mesh litterbags after 12 months of decomposition was 45.95 (± 0.80)% and 42.39 (± 0.87)% in pool and upland, respectively, for the first litterbag deployment (October 2008 - October 2009). For the second litterbag deployment (October 2009 - October 2010), mass loss was 42.63 (± 0.75)% and 37.25 (± 0.70)% in pool and upland, respectively. Mass loss from fine mesh litterbags was significantly greater in pool than upland habitats in both deployment years (p<0.0001; Figure 8, Table 4). Mass loss was not significantly different between mesh sizes within either habitat type in both deployment years.
Figure 7. The effect of vernal pool characteristics on litter mass loss rates

The litter mass loss rate (k, % day$^{-1}$) after 12 months (a: days inundated; b: water temperature) and 24 months (c: days inundated) of decomposition. Twelve month loss rates were significantly associated with hydroperiod and water temperature (p<0.01, R$^2$=0.30). Twenty-four month loss rates were significantly associated with hydroperiod (p<0.01, R$^2$=0.27). Loss rates were calculated for 24 vernal pools in a central Ontario mixed-wood forest. The relationship between mass loss rate and pool characteristics was determined by stepwise linear regression including water capacity, hydroperiod, water temperature, pH, and dissolved oxygen (SAS, Proc Reg).
Table 3. Linear stepwise regression of litter mass loss rate across pool characteristics

The relationship between 12-month litter mass loss rate (k) from coarse mesh (3mm) litterbags in vernal pools and pool characteristics was tested. Mass loss rate was calculated as the exponential decay constant of mass loss measured at 24 sites between (a) October 2008 and October 2009 and (b) October 2008 to October 2010. Pool characteristics included were hydroperiod (days flooded) flooding frequency (number of dry-down events) between October 2008 and October 2009, as well as the October 2009 water temperature, pH, and dissolved oxygen. The relationship was analyzed using a forwards stepwise linear regression (SAS 9.2, Proc Reg, entry=0.15, stay=0.10).

(a)

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<td>Days Inundated</td>
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<td>8.23x10^-7</td>
<td>3.92x10^-8</td>
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<tr>
<td>Corrected Total</td>
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(b)

Fixed Effects

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<td>23</td>
<td>1.10x10^-6</td>
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</table>
**Figure 8.** The effect of litterbag mesh size on litter mass loss

The litter mass loss (± SE) after 12 months of decomposition across habitats (pool, upland), mesh size (coarse=3mm, fine=0.5mm), and deployment year (2008, 2009). Fine-mesh litterbags differed significantly between pool and upland microhabitats in both deployment years (p<0.05). Significantly different pairs are indicated by different line styles. Litter mass loss is expressed as a percent of the initial oven-dry litter mass. Decomposition was measured at 24 sites containing vernal pools in a central Ontario mixed-wood forest.
Table 4. Effect of litterbag mesh size on 12 month mass loss

Comparison of litter mass loss across habitat type (pool, upland), mesh size (coarse 3mm, fine 0.5mm), and site for litterbags deployed in October (a) 2008 and (b) 2009. Mass loss was measured at 24 sites containing two habitat types (pool, upland) recovered after 12 months. A fully-crossed mixed model analysis was used (SAS 9.2, ProcMixed). Mesh size was treated as a fixed effect. Site and year were treated as random effects.

(a)

Covariance Parameter Estimates

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<th>Standard Error</th>
<th>Z Value</th>
<th>Pr &gt; Z</th>
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<td>2.56</td>
<td>2.26</td>
<td>0.012</td>
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<td>Residual</td>
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<td>&lt;0.0001</td>
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Fixed Effects

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<td>1.99</td>
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(b)

Covariance Parameter Estimates

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<th>Pr &gt; Z</th>
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Fixed Effects

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<td>Mesh Size</td>
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<td>Habitat x Mesh Size</td>
<td>1</td>
<td>72</td>
<td>1.86</td>
<td>0.18</td>
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3.2.4 Carbon, nitrogen and ash content of litter

The carbon, nitrogen, and ash content (expressed as percent of ash-free dry mass), as well as carbon to nitrogen ratio, of leaf litter were determined for litter samples taken from coarse-mesh (3mm) litterbags deployed in October 2008 and recovered after 0 and 12 months of decomposition (Figure 9, Tables 5 and 6) Carbon content did not differ significantly between recovery times (0, 12 months), or between habitat (pool, upland). The nitrogen and ash content increased significantly between 0 months and 12 months of decomposition (p<0.0001), but did not differ between pool and upland at either time. The C:N ratio decreased significantly between decomposition time (0, 12 months) but did not differ between habitat (pool, upland) (p<0.0001).
Figure 9. Carbon, nitrogen, and ash content of litter

The carbon (a), nitrogen (b), and ash (c) content of litter, as well as the carbon to nitrogen ratio (d) within coarse-mesh (3mm) litterbags across habitats (pool, upland) and decomposition time (0 months, 12 months). Significantly different values are not covered by the same line. Carbon content of litter did not differ significantly between either decomposition time or habitat. The nitrogen and ash content of litter increased from 0 to 12 months of decomposition (p<0.0001). The carbon to nitrogen ratio decreased in the same period of time (p<0.0001). Nutrient content was expressed as a percent of oven-dry litter mass. Nutrient content was determined from litter decomposition measured at 24 sites containing vernal pools in a central Ontario mixed-wood forest.
Table 5. Nutrient content of litter at pool and upland habitats

Carbon (C), nitrogen (N), ash content, as well as carbon to nitrogen ratio (C:N) of litter within coarse-mesh (3mm) litterbags across habitats (pool, upland) and decomposition time (0 months, 12 months).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unit</th>
<th>0 Months</th>
<th></th>
<th>12 Months</th>
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<tr>
<td></td>
<td></td>
<td>Upland</td>
<td>Pool</td>
<td>Upland</td>
<td>Pool</td>
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<tr>
<td>Average litter mass (g)</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>5.5</td>
<td>5.5</td>
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<tr>
<td>Carbon (C)</td>
<td>%</td>
<td>52.7</td>
<td>53.2</td>
<td>53.5</td>
<td>54.1</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td>5.27</td>
<td>5.32</td>
<td>2.94</td>
<td>2.96</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>%</td>
<td>0.4</td>
<td>0.3</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td>0.04</td>
<td>0.03</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>3.8</td>
<td>4.3</td>
<td>6.3</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td>0.38</td>
<td>0.43</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>C:N</td>
<td>X:1</td>
<td>178.3</td>
<td>211.6</td>
<td>41.0</td>
<td>39.1</td>
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Table 6. Comparison of litter nutrient content between recovery time and habitat type

Comparison of C (a), N (b), ash (c) content as well as C/N ratio (d) of leaf litter between
decomposition time (0 months, 12 months) and habitat type (pool, upland). Nutrient
content was expressed as a percent of ash-free dry mass. Comparison was carried out
with a mixed model analysis. Time and habitat were treated as fixed effects and site as a

(a) Carbon %

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<td>4.26</td>
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<td>2.77</td>
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<td>0.72</td>
<td>0.72</td>
<td>0.48</td>
<td>0.49</td>
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$R^2 = 0.051$

(b) Nitrogen %

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<td>0.0072</td>
<td>0.37</td>
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<td>Time*Habitat</td>
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<td>0.075</td>
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<td>0.020</td>
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<td>25.18</td>
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$R^2 = 0.93$
(c) Ash %

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<td>3.22x10^-4</td>
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<td>5.45x10^-6</td>
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$$R^2 = 0.37$$

(d) C/N ratio

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<td>6.18x10^3</td>
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<tr>
<td>Time * Habitat (1)</td>
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<td>7.72x10^3</td>
<td>7.72x10^3</td>
<td>1.52</td>
<td>0.22</td>
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<tr>
<td>Error</td>
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<td>Corrected Total</td>
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$$R^2 = 0.56$$
3.3 Laboratory leaching experiments

3.3.1 Experiment 1: Time course of mass loss from unconfined litter

The litter mass loss decreased exponentially over 28 days of soaking within leaching microcosms (Figure 10a). Mass loss increased from 18.1 (± 0.7) % after 3.5 days of soaking to 33.8 (± 0.8) % after 28 days. The litter mass loss rate (k) for 28 days of decomposition was 1.30 x 10^{-2} % day^{-1}.

3.3.2 Experiment 2: Time course of mass loss from confined and unconfined litter

Mass loss followed a negative exponential curve over 42 days of soaking for both confined and unconfined litter within leaching microcosms (Figure 10b, Table 7). After 42 days, mass loss was 31.9 (± 1.7) % and 19.3 (±2.0) % for unconfined and confined litter, respectively. The litter mass loss rate (k) for 42 days of decomposition was 7.34 x 10^{-3} % day^{-1} for the unconfined litter and 3.21 x 10^{-3} % day^{-1} for the litter confined in litterbags. Litter mass loss differed significantly between confinement (loose, litterbag) after 7, 14, and 42 days of soaking (p<0.01). The mass loss was slightly greater from loose litter than in the litter confined in a litterbag.

3.3.3 Experiment 3: Effect of water temperature and on litter mass loss

Mass loss after 28 days was not affected by temperature (5°C, 20°C) (p>0.05).

3.3.2 Experiment 4: Effect of leachate concentration and litter confinement on mass loss

The litter mass loss was significantly greater in unconfined litter than from litterbags, regardless of leachate concentration (p<0.0001, Figure 11, Table 8).
Figure 10. Time course of mass loss in microcosms

The litter mass loss (± SE) over time within leaching microcosms for unconfined litter over 28 days of soaking (a) and confined and unconfined litterbags over 42 days of soaking (b). The litter mass remaining over time was significantly explained by a negative exponential model for all treatments (p<0.0001). Litter mass loss was significantly faster from unconfined litter than confined litter. Significantly different pairs are marked by (*). Mass loss was expressed as a percent of the initial oven-dry litter mass. Each microcosm consisted of a 21 L plastic container containing naturally abscised air-dried Red Maple (Acer rubrum L.) litter soaked in untreated well-water at 5°C. Litter confinement treatment consisted of litter either confined within a coarse-mesh (3mm) litterbag, or loose within the microcosm.
Table 7. Comparison of mass loss across litter confinement and time

Comparison of litter mass loss over time across litter confinement type (litterbag, unconfined) within leaching microcosms. The litter mass remaining was compared across removal times and confinement type using a fully-crossed mixed model analysis. Each microcosm consisted of a 21 L plastic container containing naturally abscised air-dried Red Maple (*Acer rubrum* L.) litter soaked in untreated well-water at 5°C for 28 days.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>P &lt; F</th>
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</tr>
<tr>
<td>Error</td>
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<td>1.19x10¹</td>
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<td></td>
</tr>
<tr>
<td>Corrected Total</td>
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<td>4.51x10³</td>
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</table>

\[ R^2 = 0.93 \]
**Figure 11.** Effect of leachate concentration and litter confinement on mass loss

Litter mass loss (± SE) after 28 days within leaching microcosms was significantly greater from unconfined than confined litter, regardless of leachate concentration (0%, 50%, 100%) (p<0.001, R²=0.64). Significantly different values are indicated by different line styles. Litter confinement treatment consisted of litter either confined within a coarse-mesh (3mm) litterbag, or unconfined within the microcosm. Leachate consisted of the water recovered from microcosms incubated with litter at 5°C for 28 days. The leachate was added to microcosms at a rate of 0%, 50%, and 100% of the total liquid, with the remainder being untreated well-water as above. Each microcosm consisted of a 21 L plastic container containing naturally abscised air-dried Red Maple (*Acer rubrum* L.) litter soaked in untreated well-water at 5°C.
Table 8. Comparison of mass loss between leachate concentration and litter

Comparison of litter mass loss after 28 days of soaking between litter confinement type (litterbag, unconfined) and leachate concentration (0%, 50%, 100%) within leaching microcosms. The litter mass remaining was compared across removal confinement type and leachate concentration using a fully-crossed mixed model analysis. Each microcosm consisted of a 21 L plastic container containing naturally abscised air-dried Red Maple (*Acer rubrum* L.) litter soaked in untreated well-water at 5°C for 28 days.

<table>
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<tr>
<th>Source</th>
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<th>Mean Square</th>
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<td>&lt;.0001</td>
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<td>0.06</td>
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<td>Corrected Total</td>
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<td>761.5</td>
<td></td>
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$R^2 = 0.64$
CHAPTER FOUR

DISCUSSION

The difference in litter mass loss between pool and upland was not consistent over time (Figure 6). Mass loss was greater in vernal pools than adjacent uplands after 6 months of decomposition, but that difference declined over time. There was no difference in mass losses between pool and upland habitats after 12 months of decomposition, and after 24 months mass losses were greater in upland than pool habitats. Overall, litter mass loss rates were slightly greater in pool than upland habitats during the first two years of litter decomposition. During the first 6 months of decomposition, vernal pools of Central Ontario are 'hot spots' of decomposition. After the first 12 and 24 months of decomposition vernal pools are likely not 'hot spots' of decomposition, and may reduce decomposition rates, compared to adjacent uplands. Mass losses are likely driven by both physical and biological mechanisms. The relative importance of each mechanism to decomposition is likely to change over time, therefore each stage will be considered separately.

4.1 Early decomposition

In both years mass loss after the initial 6 months of decomposition was rapid in pool and upland habitats, but significantly higher within vernal pools (Figure 6). It is likely that in the present study a large proportion of early mass loss at both pool and upland habitats, as well as differences between them, is probably attributable to leaching. In the leaching experiment using microcosms (Figure 10) up to 33.7% of the original mass was lost within the first 28 days of inundation Leaching losses from decomposing litter are known
to be significant. When exposed to simulated rainfall Corrigan (2008) found that sugar maple litter lost up to 25% of its original mass after 14 days. In field experiments in forested uplands losses attributed to leaching ranged from 2% to 29% (Lousier and Parkinson, 1976; Moore, 1983; Salamanca et al., 1997; Ellis et al., 1999). Leaching caused losses of 30% to 60% after 6 months of decomposition in streams and wetlands of northeastern North America (Petersen and Cummins, 1974; Mackay and Kersey, 1985; Battle and Golladay, 2001; Hutchens and Wallace, 2002; Palik and Batzer, 2006). When inundated in a laboratory setting, Day (1983) observed losses of up to 30% initial mass for red maple litter after 3 days, and found that mangrove (*Rhizophora mangle* L.) litter lost up to 31% after 21 days (Davis et al., 2003). In studies in which mass losses at upland and wetland habitats were both observed, leaching losses were greater from the wetland than uplands within the same study sites (Day, 1982; Neckles and Neill, 1994; Hutchens and Wallace, 2002; Palik and Batzer, 2006). Litter mass loss in the present study was similar to previously observed range after 6 months of decomposition in both pool and upland. As with previous studies of aquatic and terrestrial forested habitats, litter mass loss after 6 months in this study was slightly greater in inundated environments.

Microbial metabolism may also have contributed to the differences in litter mass loss between pool and upland observed after 6 months of decomposition (Shure et al., 1986; Kelley and Jack, 2002). Microbial metabolic rates are higher in well-oxygenated aquatic environments than terrestrial environments (Brinson, 1977). If oxygen concentrations were not limiting within the vernal pools observed in this study, microbial metabolism could have contributed to the relatively greater mass loss in vernal pools. In a
Pennsylvania vernal pool between October and December studied by Inkley and Wissinger (2008) higher fungal and bacterial biomass was observed on litter under continuous inundation, compared with litter exposed to multiple wet-dry cycles. However, in contrast to these previous studies, it is unlikely that microbial activity greatly impacted early litter mass loss in vernal pools in the current study. Mass losses from observed leaching microcosms was equivalent to almost 90% of mass loss observed in field suggesting that microbial decomposition could have accounted for at most about 10% of early mass loss (Figure 6, 10).

4.2 Decomposition after 1 year
After one year mass losses did not differ between pool and upland habitats. This contrasts with several studies of seasonal wetlands in which first-year litter mass losses were found to be greater in the wetland than adjacent non-flooding upland (Peterson and Rolfe, 1982; Shure et al., 1986; Neckles and Neill, 1994; Ellis et al., 1999; Battle and Golladay, 2001; Kelley and Jack, 2002; Schmidt et al., 2002). A single experiment on prairie potholes reported lower mass losses within wetlands than adjacent upland (Neckles and Neill, 1994). This suggests that vernal pools within the present study may differ from other seasonal wetlands in either one or both hydrological and biological mechanisms underlying decomposition.

In the present study, mass loss in both pool and upland followed a negative exponential model, with rapid initial mass loss followed by a much slower rate of loss (Figure 6). Previous studies of mass loss in seasonal wetlands have found two divergent trends of mass loss over time. Similarly to the present findings, a negative exponential pattern of
leaf litter mass loss through the first year of decomposition was found in Minnesota vernal pools (Palik and Batzer, 2006), New Mexico riparian forests (Ellis et al., 1999), and Manitoba prairie pothole wetlands (Neckles and Neill, 1994). These temporary wetlands typically had one annual inundation event the duration of which ranged from 1 to 5 months. Mass losses over time in both pool and upland habitats in the present study were similar to those observed wetlands with a single annual inundation. In contrast, leaf litter mass losses were linear throughout the first year of decomposition within and adjacent to seasonal wetlands in Georgia (Battle and Golladay, 2001), Illinois (Peterson and Rolfe, 1982), and Louisiana (Day, 1982; Conner and Day, 1991). Many of these locations had multiple inundation and dry-down cycles per season (Peterson and Rolfe, 1982; Shure et al., 1986; Battle and Golladay, 2001). The differences in pattern of mass loss observed in these studies are unlikely to be due to differences in regional climates or wetland type, as similarities persist across the entirety of Mid-Western and south eastern North America (Shure et al., 1986; Palik and Batzer, 2006) and floodplain forests, coastal plains, and isolated swamps (Day, 1982; Peterson and Rolfe, 1982; Battle and Golladay, 2001).

Previous studies have proposed that differences in mass loss between pools may be driven by differences in pool hydrology and its effect on oxygen content (Lockaby et al., 1996; Baldwin and Mitchell, 2000; Battle and Golladay, 2001; Palik and Batzer, 2006). Persistent flooding leads to greater mass losses in wetlands when water is well-oxygenated but lower losses when water is poorly-oxygenated (Neckles and Neill, 1994; Palik and Batzer, 2006). Fluctuating inundation with multiple dry-down events increases water oxygen content and moisture content (Shure et al., 1986; Kelley and Jack, 2002).
Lack of difference between mass loss after 1 year in vernal pools and upland of the current study may be due to low oxygen content in pools, through slowed decomposition rates due to low microbial activity. This is supported by the pattern of decreasing dissolved oxygen over time (Figure 5). This pattern indicates a shift towards increasingly anaerobic conditions (Day, 1982; Reddy and D’Angelo, 1994). Similarly to other low-oxygen seasonal wetlands, litter mass loss was not greater from vernal pools than adjacent uplands within this study, likely due to anaerobic conditions.

This study found that litter mass loss rates after 1 year of decomposition were positively influenced by hydroperiod, but not number of dry-down events, (Figure 7). In contrast, previous studies have found that longer hydroperiods led to lower decomposition rates in low oxygen systems (Neckles and Neill, 1994; Palik and Batzer, 2006). The results from this study suggest that the importance of hydroperiod to leaf litter decomposition in vernal pools may be greater than its control of oxygen content. Hydroperiod length may influence other decomposition mechanisms, such as leaching or macroinvertebrate activity.

Mass losses from fine mesh litterbags after 12 months of decomposition was significantly greater at pool habitats compared to upland habitats (Figure 8). The pool environment may have influenced mass loss from fine mesh litterbags differently between pool and upland habitats through leaching, moisture content, or oxygen availability. Fine mesh litterbags, irrespective of environmental conditions, have been observed to retain moisture within litter longer into dry periods (Lousier and Parkinson, 1976). This may increase microbial activity, therefore mass loss, in fine mesh litterbags located in pools compared to the adjacent upland (St. John, 1980).
The carbon, nitrogen, and ash contents of litter placed in coarse mesh litterbags did not differ between pool and upland habitats after 12 months of decomposition. As litter chemistry was tested on litter from coarse mesh litterbags only, differences in chemical content of decomposition between pools and upland that may have been affected by mesh size were not determined.

### 4.3 Decomposition after 2 years

After 2 years of decomposition litter mass losses were slightly, but significantly, lower in the pools compared to the upland habitat. Similar patterns of mass loss were observed in sub-surface decomposition of litter in a prairie pothole wetland, where hypoxic conditions were present (Neckles and Neill, 1994). In contrast, in both southern floodplain forests and northern prairie marshes litter decomposition at the soil surface was more rapid in inundated areas, compared to adjacent uplands (Neckles and Neill, 1994; Lockaby et al., 1996). Previous studies have proposed that hydroregimes which limit oxygen availability lead to reduced mass losses, even after 2 years, due to reduced fungal and bacterial activity (Baker et al., 2001). Similar to previous studies, the lower mass loss in pools after 2 years of decomposition in the present study was likely limited by oxygen availability.

Mass loss rates within vernal pools after 2 years of decomposition were positively related to hydroperiod duration (Figure 7). This contrasts with previous studies, in which longer duration of flooding in temporary wetlands was associated with a decrease litter mass loss during the second year of decomposition (Neckles and Neill, 1994; Baker et al., 2001). The typical negative effect of hydroperiod length on decomposition rate is
supported by evidence that continuously inundated locations develop anaerobic conditions over time, therefore limiting bacterial and fungal activity (Neckles and Neill, 1994; Mitsch and Gosselink, 2000). The positive effect of hydroperiod length on decomposition in the present study is likely not related to oxygen content, and may actually be compensating for reduced microbial decomposition under low oxygen conditions. Hydroperiod length likely positively influences other decomposition mechanisms, such as leaching and/or macroinvertebrate activity. The positive influence of hydroperiod on mass loss rates in vernal pools after 2 years of decomposition is contrary to the relationship between hydroperiod and decomposition in other temporary wetlands.

4.4 Vernal Pool Characterization

The locations of the vernal pools identified within this study area were clumped within the landscape (Figure 2, Appendix 3). This supports previous studies, in which vernal pool spatial distribution was non-uniform (Lathrop et al., 2005; Van Meter et al., 2008). Vernal pool distribution is influenced by surficial geology and hydrology, land cover type, and land use (Palik et al., 2003; Grant, 2005). Although the relationships between landscape characteristics and vernal pool distribution are still poorly understood, shallow slopes seem to be a consistent indicator of vernal pool occurrence in several jurisdictions. Vernal pools in our study (Appendix 1), as well as pools in Northern Ontario (Creed et al., 2003) and Massachusetts (Grant, 2005) occur most frequently on flat or gently sloping ground. The average at pool slope in this study, 2.8°, was shallower than the 3.17° mean slope reported for Massachusetts (Grant, 2005). Vernal pools in the present
study, as with previously studied vernal pools, tend to be clumped on the landscape in
areas of low slope.

The vernal pool density observed in this study was similar to that found in other field
surveys in northeastern North America. Methodology has a significant effect on density
measures. For example, remote sensing techniques tend to generate lower estimates of
pool densities. Vernal pool density determined by field surveys across 11 sites
throughout New England ranged from 23.1 to 49.5 pools km$^{-2}$, with a mean of 30.6 pools
km$^{-2}$ (Van Meter et al., 2008). In contrast pool densities determined by remote sensing
analyses (primarily air photo interpretation) in New England ranged from 1.1 pools km$^{-2}$
to 13.5 pools km$^{-2}$ (Brooks et al., 1998; Calhoun et al., 2003; Palik et al., 2003;
Mckinney and Charpentier, 2009). The lower estimates based on remote sensing
techniques likely result from high rates of omission, which have been shown to range
from 30% (Lathrop et al., 2005) to 350% (Calhoun et al., 2003). Pools with a surface
areas of 200 m$^2$ or less may be systematically excluded from remote sensing analyses
(Brooks et al., 1998; Calhoun et al., 2003; Lathrop et al., 2005). These pools tend to have
a higher densities on the landscape (Brooks et al., 1998) are often not visible in aerial
photos. To obtain more accurate and unbiased estimates field surveys are required for
regions containing pools with small surface areas such as the present study area.
However, aerial surveys may still be effective in areas where larger pools predominate.
Vernal pools density within the present study was similar to pool densities found in field
studies, however much greater than densities found via remote sensing.
The mean hydroperiod of vernal pools in this study was slightly longer than that of pools
studied previously in Massachusetts (284 days) (Brooks, 2000). New England pools
tended to dry out between late June and early August (Brooks, 2000; Skidds and Golet, 2005; Baldwin and Calhoun, 2006), around the same time as the pools in the present study. The longer mean hydroperiod is the result of the earlier fall re-filling dates. At our study site, pools refilled between October 1st to October 15th as compared to Massachusetts where pools refilled over a longer period October 1st to December 6th (Brooks, 2000). The vernal pools in our study area also tended to attain their maximum depth in the fall (65% of pools), whereas most other pools described in the literature for northeastern North America reached their maximum depth in spring (e.g. Rowe and Dunson, 1993; Baldwin et al., 2006). The earlier re-fill dates may be due to climatic factors such as greater autumn precipitation or lower water deficits. Vernal pools at the end of the summer in this study showed a high concordance between the water depth and precipitation patterns (Figure 5a). This suggests that filling of the pools in our study area is driven by precipitation. The hydroperiod of vernal pools within the study area were slightly longer than those found elsewhere, likely due to their earlier re-filling dates.

The vernal pools in our study were smaller in both maximum depth and surface area than pools in New England. The mean water depth at maximum capacity was 2 to 3 times shallower than depths reported for pools in New England (Brooks, 2005; Skidds and Golet, 2005; Baldwin and Calhoun, 2006) and Minnesota (Palik and Batzer, 2006). The mean surface areas at maximum capacity were an order of magnitude smaller than those of pools in New England (Brooks et al., 1998; Skidds and Golet, 2005; Baldwin and Calhoun, 2006). These hydrological differences may reflect differences in local topography or methodological differences between studies. If smaller pools were excluded in previous studies, than estimates of depth and surface area would be expected
to be greater. The differences could also indicate underlying morphological differences between the pools in this study and those described in previous studies. Vernal pools within the study area tended to be smaller both in extent and depth than pools found previously in New England.

Water pH and temperature values measured within pools in this study differed from pools in other regions. The mean pH of pools in this study was 4.3, lower than the pH reported for pools in Rhode Island (5.29) (Skidds and Golet, 2005) and Pennsylvania (4.67) (Rowe and Dunson, 1993). The more acidic conditions in this study may reflect differences in vegetation and/or differences in underlying surficial geology. The mean overall temperature of pools in this study (12.4°C) was slightly higher than temperatures observed for pools in Pennsylvania (9.0°C) (Rowe and Dunson, 1993). This temperature difference is likely due to the smaller size and lower heat capacity of the pools observed in this study. Dissolved oxygen levels have not been measured in previous studies of vernal pools. In the present study, vernal pools tended to have lower water pH and higher water temperature than pools previously identified. These might relate to differences in overstory species or basin morphology.

4.5 Conclusions

Vernal pools of central Ontario represent 'hot spots' of decomposition up to 6 months of decomposition. After the first 12 and 24 months of decomposition vernal pools are likely not 'hot spots' of decomposition, and may have slower litter decomposition rates, compared to adjacent uplands. Evidence from this study suggests that after initial high leaching losses, litter in vernal pools actually decomposes at a slower rate compared to
litter in adjacent upland. Initial leaching loss is rapid, and greater in pool than upland, with losses largely due to physical mechanisms. Long duration of inundation, coupled with few dry-down events, creates anaerobic conditions within submerged litter, therefore likely limiting microbial activity and reducing mass loss due to biological mechanisms.

The distribution of vernal pools within the present study area is similar to previous studies. The vernal pools themselves, however, tend to be smaller, warmer, more acidic, and are inundated longer than pools elsewhere. Therefore, vernal pools within the mixedwood forest of central Ontario under study represent a unique type of vernal pool not yet common in the seasonal wetland literature.

The predicted impact of climate change on vernal pools hydrology is to shorten hydroperiods and increase frequency of dry-down events (Brooks, 2009). In vernal pools of central Ontario, this shift may result in faster decomposition rates as aerobic conditions are maintained longer within submerged litter. This shift will decrease the overall rate of carbon storage within forested soils due to the cumulative effect across all vernal pools in a landscape. The effect of climate change on landscape level carbon cycling will be greater in areas with greater densities of vernal pools.

This experiment could have been improved by a greater scope and longer time frame. A full carbon budget for vernal pools in the study area including inputs and outputs could have been constructed. This would have allowed a more detailed assessment of the mechanisms underlying differences in mass loss between pool and upland. The full schedule of litterbag sampling and pool monitoring used in the first 12 months of this study could have been extended to 24 months. This would have allowed for a better
understanding of mass loss dynamics within vernal pools and the underlying mechanisms up to 24 months of decomposition.

Hydrology is the key variable influencing both physical and biological decomposition mechanisms within vernal pools. However, we still do not know enough about the effects of hydrology on decomposition mechanisms within vernal pools to make specific and focused predictions of the response of decomposition processes to climate change. The relationship between hydrology and oxygen availability needs to be quantified in order to validate the current assumptions regarding the influence of hydroperiod and dry-down events on dissolved oxygen. The relative contribution of different mechanisms of decomposition, and how each might be affected by hydrology, is needed to allow predictions of decomposition under changing pool hydrology. Finally, the distribution and extent of vernal pools in Canada needs to be assessed, in order to quantify the relative importance of these small ecosystems to the larger forested landscape.

A picture is beginning to emerge of the relationships between hydrology and decomposition within vernal pools. This picture, however, is not yet complete. More work is needed to understand the mechanisms underlying decomposition processes in vernal pools. From this knowledge, the importance of these seasonal wetlands to the forest carbon cycle can be assessed, and potential changes to this role due to climate change can be predicted.
REFERENCES CITED


Ryder, D., Horwitz, P., 1995. Seasonal water regimes and leaf litter processing in a wetland on the Swan Coastal Plain, Western Australia. Marine and Freshwater Research 46, 1077.


Salonius, P.O., 1981. Metabolic capabilities of forest soil microbial populations with reduced species diversity. Soil Biology and Biochemistry 13, 1-10.


Schmidt, J.M., Daniels, W.L., Aust, W.M., Cairns, J., 2002. Litter Decomposition in Created and Adjacent Forested Wetlands of the Coastal Plain of Virginia Litter De-
composition in Created and Adjacent Forested Wetlands of the Coastal Plain of Virginia. City.


### APPENDICES

**Appendix One**

**Vernal Pool Characterization Summary**

**Table 9.** Characteristics of vernal pools within the study area

Characteristics of vernal pools measured upon initial survey. Permanent wetlands initially identified have been excluded.

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Table 10. Characteristics of vernal pools used for field experiment

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Appendix Two

Litterbag Method Report

Quantifying Leaf Litter Decomposition through the Litterbag Method

By:

Kirsten V. Otis

191264

December 21, 2010

For:

Jonathan Schmidt

ENVB*6452 Topics in Environmental Biology
Introduction

Decomposition, the physical and chemical breakdown of organic matter, is an incredibly complex process, involving abiotic, botanical, and faunal factors which interact over time resulting in changes of mass, carbon, and nutrient content in decomposing litter (Seastedt 1984). Understanding decomposition, and its many intricacies, is crucial to accurately predicting nutrient and carbon cycling in variable and increasingly impacted environments over time and space. The litterbag method, in which known amounts and types of organic litter are confined in a mesh enclosure and exposed to a environmental conditions (Cotrufo et al. 2010), allows researchers to simultaneously track the changes in litter samples throughout the decomposition process and expose that litter to agents of decomposition such as moisture, microorganisms, and invertebrates (Falconer et al. 1933, Bocock and Gilbert 1957). The experimental parameters which define the litterbag method are litterbag design, including material, dimensions, and litter substrate, sampling design, including deployment/retrieval timelines, exposure environment, and response measurement, as well as the treatment design, including environment, substrate, and/or decomposer communities (Seastedt 1984, Kampichler and Bruckner 2009, Cotrufo et al. 2010). Initially developed to determine mass loss from the leaf litter layer in forests under natural conditions (Falconer et al. 1933), the litterbag method provides a simple experimental framework which is easily adapted to suit the diversity of locations and conditions under which decomposition occurs (Aerts 1997, Gholz et al. 2000). The litterbag method, however, is also limited by this flexibility, as it currently lacks methods standardization, outside of large-scale collaborative experiments (Gholz et al. 2000, Moore et al. 2006), and the experimental effect of different method
variations are largely unknown (Seastedt 1984, Kampichler and Bruckner 2009, Cotrufo et al. 2010). The following is a review of the breadth of approaches taken in the implementation of the litterbag method to organic litter decomposition, as well as a critical assessment of the conclusions drawn from these methods.

**History of Litterbag Studies**

Litter confinement studies were initially developed to address botanical hypothesis, investigating how the litter layer changed over time, in response to natural conditions in the forest floor. Primarily observational studies, these early investigations allowed for development of hypotheses regarding the conditions which influence decomposition, including climate, litter type, and site characteristics (Falconer et al. 1933, Gustafson 1943). Confinement was achieved through open-top mesh baskets of galvanized iron placed in contact with the soil surface and filled with the top litter layer of equal surface area, and response was measured as oven-dry mass loss over time (Falconer et al. 1933, Gustafson 1943). This method, however, allowed for additional litter input to the baskets, litter loss due to fragmentation, and soil input, leading to unknown errors in mass loss measurements (Gustafson 1943). The technique was refined to increase external validity, through the use of nylon mesh bags that could better integrate into the litter layer, and increase strength of conclusions, through the use of fully-crossed and replicated experimental designs (Bocock and Gilbert 1957). Through statistical tests such as ANOVA, the response of oven dry mass loss from litter bags was found to be significantly influenced by site characteristics, including soil type (Bocock and Gilbert 1957), elevation and overstory species, and species of decomposing litter (Bocock 1964). Early studies also investigated differences between confined and unconfined
decomposition rates, finding that confined litter lost mass at a slower rate, compared to unconfined litter, and concluded that the litterbag method represents relative comparisons between standardized conditions, rather than absolute measure of mass loss (Bocock and Gilbert 1957).

As the use of litterbags to investigate decomposition increased, the methodology applied to them diversified to address more specific hypotheses regarding the potential influences identified in observational studies – climate (Aerts 1997), litter substrate (Lousier and Parkinson 1976, Gartner and Cardon 2004), and decomposer community (Kampichler and Bruckner 2009). Exposure environments were characterized by temperature, precipitation (Berg et al. 1993), and actual evapotranspiration (AET) (Meentemeyer 1978, Berg et al. 1993), often between sites chosen to span a range of climatic conditions (Day 1982), in order to explain site-to-site differences in decomposition rates due to climate (Aerts 1997). Chemical content of decomposing litter was determined a priori, in order to link the initial chemistry of organic matter to subsequent mass loss and chemical changes over time (Lousier and Parkinson 1976). Community composition within the litterbags was surveyed (Bocock and Gilbert 1957), and community composition was limited by decreasing mesh size or applying chemical treatments (Curry 1969a), in order to determine community changes over time and the relative contribution of abiotic, microorganism, and invertebrate activity to decomposition (Seastedt 1984). Response measurements were also expanded, from simple oven-dry mass loss to include specific chemical changes within the litter, such as carbon, nitrogen (Bocock 1964, Anderson 1973), lignin (Curry 1969a), as well as elements such as Mg, Na, Ca, and S (Gosz et al. 1972, Lousier and Parkinson 1976).
increase in specific measurements of both independent and dependant variables allowed for specific, numerical hypotheses regarding the relationship between decomposition and climate, substrate, and community, to be tested using statistical tools such as multiple regression (Trofymow et al. 2002). Lab-based litterbag studies, utilizing simplified microcosms to test the effect of a single variable on decomposition (Day 1983, Taylor and Parkinson 1988), allowed for more specific cause and effect relationships to be tested, however the required homogeneity limited the external validity of findings.

Modern litterbag studies are expanding the applicability and relevancy of litterbag experiments to a ever greater range of ecosystems and environmental conditions through large-scale collaborative studies (Gholz et al. 2000), computer-based decomposition modelling (Manzoni et al. 2010), and increasingly complex treatment designs (Gartner and Cardon 2004). Long-term, large-scale collaborative decomposition studies, such as the Canadian Intersite Decomposition Experiment (CIDET) which spans 6 years and 21 sites across Canada (Moore et al. 2006) as well as the Long-Term Intersite Decomposition Experiment (LIDET) which spans 10 years and 28 sites across North and Central America (Gholz et al. 2000), allow for comparison of mass and nutrient changes within standardized decomposition units over a wide range of environmental conditions. Increasing amounts of high-quality data from studies large and small, have allowed for the development of models, including CENTURY (Gholz et al. 2000) and DayCent (Cotrufo et al. 2010), which utilize process-based mathematical relationships to predict rates of decomposition and carbon cycling under changing conditions (Manzoni et al. 2010). More intricate treatment designs, as well as meta-analyses of existing data, have investigated complex interactions between multiple treatment factors, such as the effects
changing climatic conditions the role of microarthropods (Kampichler and Bruckner 2009) and litter substrate species (Hoorens et al. 2010) in decomposition dynamics. Most recently, litterbag studies have begun to address shortfalls initially observed, such as increased moisture (Kampichler and Bruckner 2009), and decreased fragmentation loss (Cotrufo et al. 2010) due to litter confinement, in order to increase the external validity of litterbag decomposition data. The litterbag method is widely used for a variety of hypotheses in numerous ecosystems worldwide, however the parameters used in each study vary just as widely. The future of the litterbag method lies in developing standardized experimental parameters, which can be applied across all habitats and hypotheses, and addresses the shortfalls of this method to further refine our understanding of decomposition dynamics.

**Litterbag Design**

Litterbag design, including material, dimensions, and litter substrate, must allow for integration of sample litter into the natural litter layer in order to approximate in-situ decomposition processes such as leaching, physical fragmentation, and chemical breakdown (Bocock and Gilbert 1957, Seastedt 1984). Materials used must be invert and long-lived, to ensure litterbags retain intact throughout the study period (Cotrufo et al. 2010). Early studies utilized galvanized iron, which was meshed to allow for natural processes access to decomposing litter, however the metal was both difficult to work with and somewhat reactive (Falconer et al. 1933, Gustafson 1943). Nylon hair nets were next used as litter containment to improve (Bocock and Gilbert 1957), as they are inert and flexible, to allow for greater ease of handling and more natural integration of sample
litter into the litter layer. Flat, two-sided design present in most subsequent studies were developed shortly thereafter (Crossley and Hoglund 1962), and constructed of various non-reactive synthetic fibres such as nylon (Anderson 1973, Stewart and Davies 1989), polyester (Gholz et al. 2000), and polypropylene (Moore et al. 2006), as well as fibreglass (Crossley and Hoglund 1962, Witkamp and Olson 1963). Surface area of litterbags utilized for decomposition studies range from: 140cm² (Anderson 1973) to 5000 cm² (Crossley and Hoglund 1962), however most are between 200cm² (10cm x 10 cm) and 800 cm² (20cm x 20cm) (Stewart and Davies 1989, Gholz et al. 2000, Moore et al. 2006). Mesh sizes also range widely, from 48um (Setala et al. 1996) to 7mm (Anderson 1973), however the most typical is 1mm (Witkamp and Olson 1963, Huang et al. 1998). Mesh size choice is a balance between allowing decomposer community access (Anderson 1973), while minimizing fragmentation loss of experimental substrate (Suffling and Smith 1974). Mesh sizes <0.5mm have been used to exclude meso and macro invertebrates, thus limiting the decomposer community within the litterbag (Curry 1969b, Anderson 1973). Composite litterbags, consisting of a coarse mesh upper (≥1mm) and fine mesh lower (<1mm), have been utilized in some studies to both allow access to macroinvertebrates, and minimize fragmentation loss (Stewart and Davies 1989, Gholz et al. 2000). For aquatic decomposition, some alternative litterbag designs have been used, including a square cage design (30x30x10cm) within a stream to prevent litter compression (Ribas et al. 2006), and a mesh bag with vertical pockets for substrate within lakes (Larmola et al. 2006).

Litter substrate used in decomposition studies, including type and preparation, must simultaneously balance external validity, by choosing a type of organic matter
representative of local litter and processing it as little as possible, and minimizing experimental error, by ensuring litter substrate is reasonably homogeneous in chemical composition, dimensions, and moisture content. The majority of litter decomposition studies utilize natural materials such as leaves or needles (Gholz et al. 2000, Moore et al. 2006), roots (Gholz et al. 2000), twigs (Falconer et al. 1933, Huang et al. 1998), and wood, often derived from the study site itself (Day 1982). The chemical heterogeneity inherent in organic litter has been reduced through experimental protocols, including use of a single species of litter (Aerts 1997), litter collection at a single site (Gholz et al. 2000, Trofymow et al. 2002), and thoroughly mixing collected litter before allocation to litterbags. Although raw litter best approximates natural litter conditions, procedures to homogenize litter moisture prior to deployment are common, and include air-drying at room temperature for 4 to 30 days with sub-sampling to determine oven-dry equivalent weight (Vitousek et al. 1994, Moore et al. 2006), or oven-drying at temperatures between 60°C and 105°C (Stewart and Davies 1989, Huang et al. 1998). Although this method increases homogeneity, air drying has been shown to have a species-specific effect on initial leaching loss from tree leaf litter, however this effect can be positive, negative, or negligible so litter species selection is important in determining litter processing techniques (Taylor and Barlocher 1996). In some studies, processed materials have been chosen over natural materials in order to limit variability between sampling units, such as cellulose sheets (Larmola et al. 2006), wooden dowels (Vitousek et al. 1994), and filter paper (Bocock 1964, Vitousek et al. 1994). The amount of litter substrate added to each bag varies from 1.4g (Anderson 1973) to 10g (Gholz et al. 2000), depending on litterbag
size and type of litter being used, and can be measured in raw, air-dry, oven-dry, or ash-free oven-dry mass.

Although litterbags have been a favoured method in decomposition studies over the past 75 years (Bradford et al. 2002), several studies have demonstrated that this method can underestimate magnitude and variability of mass lost from naturally decomposing litter (Bocock and Gilbert 1957, Witkamp and Olson 1963, Anderson 1973, {St. John} 1980, Cotrufo et al. 2010). These observed response differences have been linked to faunal effects, as litterbags can reduce access to decomposer organisms such as macroinvertebrates (Bocock and Gilbert 1957) and fungi ({St. John} 1980), and microclimate effects, as litterbags have higher moisture content (Witkamp and Olson 1963), longer drying times (Bocock 1964), and increased variability in moisture content than unconfined litter (Lousier and Parkinson 1976). In order to address the criticisms of the litterbag method, alternative methods to determine in-situ decomposition have been developed which range in complexity, cost, and applicability to a variety of experimental parameters. Leaf tethering, where petioles of whole deciduous leaves are attached to nylon threads (Witkamp and Olson 1963, Anderson 1973), allows for more natural integration of samples into the litter layer (Witkamp and Olson 1963), however its usefulness is limited to short-term studies of early decomposition due to increased comminution and fragmentation loss over litterbag incubation (Vitousek et al. 1994). The specific fate of carbon within the litter substrate, from which decomposition rate is calculated, can be tracked by introducing radioactive 13C to litter prior to exposure to natural decay conditions, then measuring the abundance of 13C in litter, soil, and CO2 efflux over time (Rubino et al. 2010). If the assumption that the experimental ecosystem
is at a steady state is made, then decomposition can be estimated by calculating annual litter turnover from litter input measured in open-top traps, and standing stock measured by collar sampling of the forest floor (Olson 1963, Cotrufo et al. 2010). These alternative methods have predicted greater rates of mass loss than the litterbag method (Witkamp and Olson 1963, Cotrufo et al. 2010, Rubino et al. 2010), however further studies are required to determine their applicability across the environmental and experimental range of litterbag use.

**Experimental Design**

**Field Studies**

Experimental design within litterbag studies, including exposure environment, sampling interval, and response measurements, reflect the hypotheses being investigated, which determines the degree of variation control, sampling detail, and type of response required. Most litterbag studies have been conducted under field conditions (Kampichler and Bruckner 2009), in a variety of ecosystems across the world, including forests (Moore et al. 2006), grasslands (Anderson 1973), wetlands (Day 1982), streams (Stewart and Davies 1989), or combinations thereof (Gholz et al. 2000). Numerous studies have sought to characterize decomposition response to a hypothesized influencing factor through litterbag deployment at multiple sites spanning a gradient of field conditions such as soil type (Bocock and Gilbert 1957), temperature (Shanks and Olson 1961), forest type (Gholz et al. 2000, Moore et al. 2006), or moisture regime (Day 1982, Battle
and Golladay 2001). Litterbags are typically arranged in close proximity to each other at each site and placed in contact with soil, removing newly fallen leaves, moss, or coarse woody debris to increase contact (Trofymow et al. 2002). When sub-treatments such as litter type (Bocock and Gilbert 1957, Schweitzer et al. 2005) or mesh size (Curry 1969b) were used, blocks of all sub-treatment variants were often included within each site. Observational studies which compare response between sites, without numerical measurements of covariant site characteristics such as temperature and precipitation (Aerts 1997), allow investigators to utilize analysis techniques such as ANOVA (Bocock and Gilbert 1957) to draw conclusions regarding the differences in decomposition between sites, however not about why the sites differ (Gustafson 1943, Crossley and Hoglund 1962). In studies where covariate parameters have been measured at each site the response of mass remaining to the covariate parameter over time can be described through stepwise multiple regression, with the best-fit models selected by R2 value (Trofymow et al. 2002). This experimental design is limited to conclusions regarding the relationship between treatment and response, and cannot make claims of direct causal relationships, as it does not exclude the possibility of an unmeasured covariate parameter which is the true driver of the response. Some in-situ environmental manipulation has been undertaken in litterbag studies, such as flooding regime (Ellis et al. 1999), however this approach is uncommon.

**Microcosm Studies**

Causal conclusions regarding litter decomposition, however, can be gained from lab-based, microcosm studies as these simplified systems allow for direct manipulation of factors difficult to modify in the field, such as environmental conditions (Day 1983,
Litter decomposition Microcosms are typically enclosed containers, from 1 L (Hobbie 1996) to 400 L (Bradford et al. 2002) under controlled environmental conditions. These microcosms range from simple systems of litter and water to determine initial leaching loss (Anderson 1973, Day 1983), to complex systems consciously designed to replicate natural soil conditions and plant communities (Setala et al. 1996, Bradford et al. 2002), in some cases containing soil and organic matter transported intact from a field site (Day 1983). Litter within microcosms is often equivalent in source, treatment, and litterbag containment to field based studies, especially if the lab component is paired with an in-situ litterbag study (Anderson 1973, Day 1983). The effects of influencing factors on decomposition can be investigated through direct manipulation of environmental conditions, such as temperature (Hobbie 1996), flooding regime (Day 1983), and day length (Setala et al. 1996), as well as community composition through sterilization of microcosm and re-introduction of a whole or part of decomposer community (Setala et al. 1996, Bradford et al. 2002). Response measurements are similar to field based litterbag study (Hobbie 1996), analyzed through ANOVA (Hobbie 1996, Bradford et al. 2002) and exponential decay models (Day 1983, Cotrufo et al. 2010), further elucidating the processes and influences of litter decomposition.

**Field Parameters**

A litterbag study typically progresses through three field stages, in which all litterbags are deployed at one time into field conditions, sub-sets of litterbags representing all treatment variants are periodically retrieved without replacement at pre-determined intervals, and finally each litterbag is analyzed for response, including mass,
carbon, and nutrient content (Kampichler and Bruckner 2009). The parameters which define each stage, however, vary greatly between studies (Kampichler and Bruckner 2009). Litterbag deployment has occurred at all times of year, however most often litterbags are deployed in autumn, in order to coincide with the natural period of greatest litter input (Lousier and Parkinson 1976). The duration of litterbag studies range from < 1 month (Stewart and Davies 1989) to > 5 years (Gholz et al. 2000), with 2 (Siedentop 1995) to 35 (Witkamp and Crossley 1966) retrieval times at intervals from 1 week (Crossley and Hoglund 1962, Stewart and Davies 1989) to 1 year (Moore et al. 2006).

From data reported in Kampichler and Bruckner (2009), it was calculated that the 30 litterbag decomposition publications utilized for their meta-analysis had a median duration of 282 days, with 86% less than 1 year, and a median sampling interval of 42.5 days with 5 retrievals times. Some studies deviate from this typical pattern, such as Crossley and Hoglund (1962), which sampled litterbags with replacement, however are quite rare.

*Response Measurements*

Response to treatment is measured on each individual bag, which represents a sampling unit, however the combined data produces time-series responses for each treatment combination. Primary response measure reported in most litterbag studies is oven-dry mass loss, which can be represented as mass change (Falconer et al. 1933), percent mass loss (Gustafson 1943), or percent mass remaining (Moore et al. 2006, Cotrufo et al. 2010) of litter mass at deployment. Moisture content at retrieval has also been calculated, by measuring wet mass of litter bags upon removal from the field, as well as after oven-drying (Crossley and Hoglund 1962, Witkamp and Olson 1963). Error
can be introduced to mass loss measurements through contamination of litterbags with soil or plant growth, and litter spillage due to handling, however procedures have been implemented in some studies in order to minimize potential errors. Increased mass, as well as inorganic carbon, due to soil infiltration can be corrected by subtracting inorganic mass from final mass, or inorganic carbon from final carbon, both determined by combustion of a sub-sample of litter within a muffle furnace at a minimum temperature of 5000°C for at least 1 hour (Schweitzer et al. 2005). Increased mass due to plant growth within the litterbag can be addressed through physical inspection and removal of the additional material (Curry 1969a), or radioisotope labelling of initial litter (Rubino et al. 2010). Spillage error, mass loss due to handling during deployment and retrieval, has been found to be an average of 4.2%, and up to 10%, of initial litter mass (Lousier and Parkinson 1976) and increases with decreasing decomposition rate (Suffling and Smith 1974). Mass loss calculations are corrected for spillage error by subtracting mass change from litterbags retrieved immediately after deployment from initial litter mass (Lousier and Parkinson 1976).

Chemical analysis of both carbon and nutrient content can be carried out on initial and post-treatment litter to determine how litter chemistry is changing over time, in response to treatment. Prior to chemical analysis litter is typically oven dried to a constant mass, with reported oven temperatures ranging from 55°C (Trofymow et al. 2002) to 105°C (Huang et al. 1998), then homogenized through a 0.2mm screen using a Wiley Mill (Trofymow et al. 2002). Total carbon content, expressed as percent of oven dry mass, can be determined by combustion using an analyzer such as the LECO CR-12 carbon system, for oven-dry litter samples to obtain total carbon or for muffled litter
samples to obtain inorganic carbon, allowing organic carbon to be calculated (Trofymow et al. 1995). Within several studies, organic carbon was further separated based on solubility properties into fats, waxes, and oils (non-polar extractables, NPE) (Trofymow et al. 2002), simple sugars and water-soluble phenolics (water-soluble extractables, WSE) (Johansson et al. 1995, Trofymow et al. 2002), cellulose (acid soluble carbohydrates, ACID) (Vitousek et al. 1994, Trofymow et al. 2002), acid insoluble lignin (Klason lignin) (Vitousek et al. 1994, Johansson et al. 1995, Trofymow et al. 2002), and ash (Trofymow et al. 1995). Although this method of determining lignin content is common, the lignin measured represents many types of lignin molecules, therefore the results from this method are imprecise, and cannot be compared with other methods of assessing lignin (Trofymow et al. 1995, Oertli et al. 2002). Analysis of nutrient content in litter, including N,P, Ca,Mg,K (Vitousek et al. 1994, Trofymow et al. 2002), S (Trofymow et al. 2002), and Mn (Johansson et al. 1995), has been accomplished through combustion (Vitousek et al. 1994, Johansson et al. 1995), acid digestion (Johansson et al. 1995), and chemical analysis (Johansson et al. 1995, Trofymow et al. 2002). Both carbon fractions and nutrients have been reported as a proportion of substrate (mg nutrient/g oven-dry substrate) (Johansson et al. 1995, Trofymow et al. 2002) and as proportion of initial nutrient content remaining (Vitousek et al. 1994). As well, carbon fractions and nutrients can be reported as a stand alone response variable variable (i.e. lignin) (Vitousek et al. 1994, Johansson et al. 1995) or as a ratio (i.e. C:N, C:P) (Vitousek et al. 1994, Trofymow et al. 2002).

The response of the decomposer community within the litterbags to treatments, and litter change over time, for both invertebrate and microorganism populations. The
invertebrate community can be monitored through destructive sampling, such as opening litterbags and hand-sorting invertebrates within (Curry 1969b). Non-destructive sampling of litter bags is also possibly by suspending drying litterbags within Tulgren funnel for meso and macro invertebrates (Crossley and Hoglund 1962, Blair et al. 1990), or a Baerman funnel for micro invertebrates such as nematodes (Vossbrinck et al. 1979, Blair et al. 1990). Bacteria abundances within post-treatment litter can be sampled through FITC and epifluorescence (Blair et al. 1990). Fungi can be sampled for total length within post-treatment litter through the agar film technique (Jones and Mollison 1948), and for total biomass through ergosterol analysis (Uchida et al. 2000).

**Treatment Design**

Litter decomposition is most often investigated through treatments of decompositional environment, through climate and soil (Aerts 1997), litter substrate, through species and combinations (Aerts and de Caluwe 1997, Gartner and Cardon 2004, Schweitzer et al. 2005), and decomposer community, through species limitation (Seastedt 1984, Kampichler and Bruckner 2009). Environmental treatments in litterbag studies seek to determine how environmental factors such as climate and soil influence decomposition. Primarily in field-based studies, environmental treatments are accomplished through measurement of decomposition at multiple sites, then characterizing differences between sites. Climate differences are measured through nearby climate stations (Falconer et al. 1933, Vossbrinck et al. 1979, Johansson et al. 1995, Trofymow et al. 2002), which provides large independent data sets from which annual and average values can be derived (Trofymow et al. 2002). Climate can be expressed as various measurements, such as temperature or precipitation (Trofymow et
al. 2002), or calculated composite values, such as actual evapotranspiration (AET) (Meentemeyer 1978, Aerts 1997). At global scales, AET has been found to be positively related to decomposition rates, and the best climatic predictor of decomposition constants (k-values) (Aerts 1997). Soil differences are measured through physical characteristics (Hobbie 1996), such as temperature and permeability, and chemical characteristics, such as nutrient content. Soil is characterized through direct soil testing and observation of at each site (Gosz et al. 1972), and treatments may also include manipulation of conditions, such adding nitrogen (Prescott 1995) or increasing ambient temperature (Hobbie 1996). Previous litterbag studies have found soil temperature positively influences decomposition (Hobbie 1996), and litter decay is more rapid at nitrogen-rich sites (Gosz et al. 1973), and under the addition of nitrogen (Prescott 1995). The main limitation of environmental treatments is that manipulation of environmental properties across large scales difficult, therefore limiting comparisons to natural site-to-site differences, or variation across small scales.

Substrate treatments in litterbag studies address how the chemical contents of the decomposing litter affects its decomposition (Aerts 1997), and the interaction effects of combining multiple litter types in one litterbag (Gartner and Cardon 2004). Substrate treatments are carried out primarily through field studies, as this treatment can be manipulated easily regardless of decompositional environment and allows changes over time to be easily monitored. Litter input characterized most often is deciduous (Trofymow et al. 2002) or coniferous (Gholz et al. 2000) leaf litter, however roots (Gholz et al. 2000), wood (Trofymow et al. 2002), or combinations thereof, are also used. Litter chemistry is characterized with same methods as carbon and nutrient response variable,
and can be utilized as a numeric independent variable, allowing for regressions of mass loss across a variety of nutrient contents across species (Vitousek et al. 1994, Johansson et al. 1995). Litter substrate consisting of a single species per litterbag, comparing decomposition between multiple species (Gholz et al. 2000), or between a single species over an environmental or community treatment gradient, are the most common treatment designs (Gartner and Cardon 2004). The specie(s) chosen for single-species treatments are often representative of the study area (Day 1982), and by reducing confounding variables, allow for strong conclusions regarding the effect of a particular species on decomposition (Aerts 1997), and guide further hypotheses on the chemical basis underlying those differences. Litterbag studies with multiple species in each litterbag can either be haphazard (Falconer et al. 1933, Ribas et al. 2006) or pre-planned (Gartner and Cardon 2004). Haphazard multiple-species litterbags often utilize natural mixtures of litter found on a site, in order to increase external validity (Ribas et al. 2006), however this approach is naive, as the unknown species mixture cannot be replicated, and conclusions reached cannot be extrapolated beyond the current study. Planned multiple-species litterbags conscious place 2 or more species together, and often include single-species litterbags of each species, with the same total litter mass as multiple-species litterbags, used as control treatments (Gartner and Cardon 2004). These studies are more representative of natural conditions, and have led to the observation that certain species, when combined, exhibit non-additive decomposition rates, resulting in greater or less mass loss than predicted.

Decomposer community treatments address how community composition of soil invertebrates and microorganisms influence decomposition, both independently and as a
complete system. In-situ community treatments are categorical, representing different segments of the litter decomposer population, delineated by site-to-site differences (Bocock and Gilbert 1957), or specific exclusion treatments using mesh size or chemical applications (Bradford et al. 2002, Kampichler and Bruckner 2009). Comparisons of decomposition between sites with observed differences in decomposer species allow conclusions to be made about site differences, and help to develop more complex hypotheses regarding the role of different segments of the decomposer community (Bocock and Gilbert 1957). The most common method to limit species community at a single site is through mesh size, which is assumed to be an analogue to body size of different litter communities (Curry 1969a, Bradford et al. 2002), Micro fauna <0.100mm; mesofauna (0.1-2mm); macro fauna (>2mm) (Swift, Heal, Anderson 1979). In practice however, mesh size varies widely and does not always conform to these size delineations. Fine mesh litterbags, intended to exclude all fauna but micro-organisms, range from 0.003mm (Curry 1969a) to 1.9mm (St. John 1980), but most often less than 1mm (Bocock 1964, Ribas et al. 2006). The exclusion, however can be incomplete, as in some studies fine mesh was unable exclude nematodes and targriades (Vossbrinck et al. 1979) as well as enchytraeids and colembola (Curry 1969b), and demonstrated a positive effect on populations of nematodes, enchytraeids, and collembolan (Curry 1969b), possibly due to favourable moisture content and exclusion of larger predators. Medium mesh litterbags, intended to allow microorganisms, mites, springtails, enchytraeids, and small invertebrates, range from 0.5mm (Curry 1969a) to 2mm (Bradford et al. 2002), and are not present in many studies. Coarse mesh litterbags, intended to allow access to all invertebrates but limit fragmentation loss, ranged from 4.7mm (Bradford et al. 2002) to
10mm (Bocock 1964) with the most common mesh size 7mm (Curry 1969a, Anderson 1973). Assumption is made that community is the only thing mesh size influences, however coarse mesh litterbag have been found to dry faster (Bocock 1964) and have a lower moisture content (Lousier and Parkinson 1976), have greater mass loss due to spillage (Suffling and Smith 1974), and greater soil infiltration, plant in growth, and leaching loss (Curry 1969a), when compared to fine mesh bags. Organisms can also be excluded through chemical treatments, such as naphthalene (Seastedt 1984, Blair et al. 1990) and c Chemical treatments are effective at lowering, but not eliminating, all faunal activity within the litterbags, and unplanned effects such as increased leaching or oxidation have been posited. There are a few microcosm based studies, which consciously manipulate decomposer community composition, therefore allowing for testing of specific, causal hypotheses regarding the influence of mesh size and decomposer community, as well as the interaction of the two, on leaf litter decomposition (Bradford et al. 2002). Currently very simple, these studies point more to flaws in the litterbag method itself, such as decreased decomposition in fine mesh bags irrespective of faunal treatment (Bradford et al. 2002), than clearly elucidating the role of decomposer community in litter decomposition.

**Conclusions**

Decomposition is a complex process, and the litterbag method used extensively to elucidate the processes and interactions underlying the physical and chemical changes in organic litter over time. The litterbag method provides a simple, flexible methodology that can be utilized in numerous environments to address environmental, substrate, and community hypotheses. The method, however, has limited in its ability to replicate all
conditions of unconfined litter and does not meet required assumptions when multiple mesh sizes are employed. More research is required to quantify the precise differences between confined and unconfined litter and the treatment effects of multiple mesh size, in order to facilitate the development of a set of standardized experimental parameters applicable to a wide range of study types. The litterbag method has served faithfully as a standard tool in the investigation of litter decomposition for over 75 years, and with continued diligence to improve on methodological errors, can continue to be a useful practice into the future.
Literature Cited


Appendix Three

Vernal Pool Prediction Analysis

Introduction

As humans expand their footprint on the natural landscape, systematic conservation planning and land management are increasingly necessary to protect environmental form and function (Cabeza et al. 2010). Modeling of species area distributions in space and time can be a key component of this planning, as it identifies areas of elevated conservation value that support high species richness as well as sensitive or threatened species (McKenney et al. 1998, Cabeza et al. 2010, Soberón 2010). These models represent spatial hypotheses of potential habitat used by the target species in order to predict species occurrence. Species area distributions are quantified by metrics such as probability of occurrence and habitat suitability, and are calculated by a variety of methods, including linear regression (Nicholls 1989, Mladenoff et al. 1995, Guisan and Zimmermann 2000, Fotheringham et al. 2002), niche factor analysis (Hirzel et al. 2002), and maximum entropy estimation (Phillips et al. 2006). Species area distribution maps representing potential occurrences are generated through Geographic Information Systems (GIS), based on modeled relationships between locations of known presence and landscape features such as climatic variables, biotic interactions, biogeographic limitations, and occupancy dynamics (McKenney et al. 1998, Mackey and Lindenmayer 2001, Hortal et al. 2010, Soberón 2010).

Species area distribution models are often developed at coarse scales, spanning 10 to 100s of km2, using widely available regional or national data such as temperature and precipitation (McKenney et al. 1998, Kadmon et al. 2003, Cabeza et al. 2010). However,
conservation implementation often occurs at medium to fine scales, usually less than 10 km2, where biologically relevant processes as well as temporal frequencies may be influenced by highly heterogeneous landscape features such as forest cover, water features, or human land use (Mackey and Lindenmayer 2001, Beale et al. 2008). In order to address this issue, a nested hierarchy approach has been proposed (McKenney et al. 1998, Palik et al. 2003), which utilizes species distribution limits identified from coarse scale analysis to spatially constrain more detailed analyses at medium and fine scales. Modeling approaches can also be applied at fine scales to predict the distribution of habitat features which are often ephemeral or difficult to find but critical in determining the distribution of the target species at smaller scales (Lindenmayer et al. 1991, Mackey and Lindenmayer 2001).

Such modeling approaches were used by Palik et al. (2003) in Minnesota and Grant (2005) in Massachusetts to predict the occurrence of temporary forested wetlands known as vernal pools, based upon variables such as climate, land cover, and geological history. Vernal pools support diverse communities of vertebrate, invertebrate, and plant species, which include numerous endemic and obligatory species, exhibit high compositional differences between pool communities, and contribute disproportionately to landscape-scale biodiversity relative to pool area or volume (Oertli et al. 2002, Palik et al. 2003, Williams et al. 2003, Colburn 2004, De Meester et al. 2005). Ephemeral wetlands are particularly well-suited for habitat modeling, as their small size, geographic isolation, and seasonal nature make them difficult to locate in traditional aerial and ground surveys (De Meester et al. 2005).
Models of vernal pool distributions have been developed through multiple logistic regression (Grant 2005), regression tree analysis (Palik et al. 2003), and topographic depression identification (Creed et al. 2003). These models integrate multiple landscape features, such as topography, surficial geology, and ecological context, into a predictive function of vernal pool distribution estimators such as occurrence, abundance, and area, within the study region. Slope has been found to be a significant predictor of vernal pool distribution. In Massachusetts 95% of photo-identified pools occurred at slopes less than 9.3° (Grant 2005) and in North-Central Ontario (Creed et al. 2003) areas with slopes less than 1.5° were correlated with vernal pool surface area. However, each regional model utilizes different prediction methods, distribution estimators, and combinations of landscape factors, making direct comparisons of predicted vernal pool distributions between regions difficult. A simple, general modeling framework is needed to facilitate inter-regional comparisons and explore the conditions and processes which lead to vernal pool formation at a landscape scale.

In this study a habitat distribution model of vernal pools was developed using a single feature, landscape slope, in order to determine if a simple statistical relationship could predict vernal pool occurrence. Landscape slope was chosen as the sole predictor variable as it has a demonstrated link to vernal pool presence, and because elevation data, from which slope can be derived, is readily available for most regions of vernal pool occurrence (Creed et al. 2003, Grant 2005). It was hypothesized that vernal pool density in Central Ontario would be significantly correlated with slope, and that slope could predict vernal pool occurrence at the landscape level.

Methods
Study Area

This study was conducted in the Frost Centre Area, approximately 24,000 hectares of provincially owned mixed-wood forest within the Great Lakes-St. Lawrence Forest Region of central Ontario, Canada (Frost Centre Working Committee 2001). The landscape within the study area consists of gently rolling uplands, with lowlands containing numerous lakes, rivers and wetlands. The upland forest is dominated by maple spp. (Acer saccharum Marsh, A. rubrum L.), oak spp. (Quercus alba L., Q. rubra L.), and pine spp. (Pinus strobus L., P. resinosa Ait.) (Burns and Honkala 1990). The Frost Centre Area is managed by the Ontario Ministry of Natural Resources for mixed use, including timber harvesting, scientific research, and recreational activities (Frost Centre Working Committee 2001).

Field Survey

Vernal pools were identified during a terrestrial field survey conducted from May to October 2008 (Figure 2). The survey was limited to areas accessible (≤ 500m) from established roads or trails and likely to contain vernal pools (slope < 10o) (Creed et al. 2003, Grant 2005). Vernal pools were defined as the occurrence of standing or seeping water lacking any overland hydrological connection. No minimum size requirement was used, due to ambiguity of maximum flooding extent (Palik et al. 2003, Colburn 2004, Grant 2005). The spatial coordinates of all vernal pool locations were recorded upon initial identification using an eTrexH handheld GPS, with an average horizontal accuracy of ± 15m (Garmin, Part No. 010-00631-00, Olathe, KS). Vernal pools were re-surveyed in late summer to verify dry-down and exclude permanent wetlands from subsequent
analyses. The field survey resulted in an unknown number of omissions due to time, weather, and terrain constraints, therefore vernal pool locations reflect a low estimate of the actual number of pools potentially on the landscape.

**Geospatial Analysis**

Vernal pool spatial coordinates were imported into Arc GIS (V9.2, Environmental Systems Research Institute Inc., Redlands, CA) as a point feature layer. The area surveyed was hand digitized into Arc GIS (V9.2, Environmental Systems Research Institute Inc., Redlands, CA) as a polygon feature layer which includes all areas thoroughly searched, as recorded by the Track Log feature of an eTrexH handheld GPS (Garmin, Part No. 010-00631-00, Olathe, KS). Other feature data layers (i.e. lakes, wetlands, roads) were obtained from the CanMap Atlas Bundle (Desktop Mapping Technologies (DMTI) Inc. 2009).

The slope data layer was generated for the survey area from a Digital Elevation Model with a 10m horizontal resolution (Water Resources Information Project (WRIP) 2002), using the SLOPE tool within Arc GIS (V9.2, Environmental Systems Research Institute Inc., Redlands, CA). A 3x3 cell moving minimum filter was applied to the slope layer in order to introduce a small degree of spatial autocorrelation (Lindsay et al. 2004). This reflects the positional error inherent to recorded GPS coordinates, ± 15m on average (Garmin 2007). All cells within the slope layer were equally and randomly allocated to either a calibration or a validation data set, and all data preparation was carried out on both sets (Guisan and Zimmermann 2000). Slope data, including vernal pool and cell count per 0.01o slope, were extracted from the slope layer.

**Data Preparation**
Slope values were reclassified into 14 categories (n) of size 0.5° starting at 0°. Each category is represented by the minimum slope (s). The slope range used includes all slopes upon which vernal pools were observed, while the category width of 0.5° reduces the number of categories with zero vernal pools. Vernal pool abundance (As), area in km² (Rs), and density in pool/km² (Ds) were calculated for each slope category. Area-weighted vernal pool abundance (Ws) was calculated (Equation 1) to account for unequal selection probability due to differential survey area (Pfeffermann et al. 1998).

\[ W_s = A_s \times \frac{\sum_{s=0}^{n} R_s}{R_s} \quad (1) \]

The total area-weighted vernal pool abundance (Wtotal; Equation 2), probability of vernal pool occurrence per slope category (Os; Equation 3), and cumulative probability of occurrence (Oi ; Equation 4) across increasing slope (i) were estimated to facilitate model fitting and prediction of vernal pool abundance at a landscape scale (Mladenoff et al. 1995).

\[ W_{total} = \sum_{s=0}^{n} W_s \quad (2) \]

\[ O_s = \frac{W_s}{W_{total}} \quad (3) \]

\[ O_i = \sum_{s=0}^{i} \frac{W_s}{W_{total}} \quad (4) \]

Statistical Analysis

The calibration data set was used to fit linear, Poisson, and logistic regression models to slope (i) and vernal pool occurrence (Oi) using SAS GENMOD procedure.
These models have been utilized in previous studies to predict habitat occurrences from asymmetric count and probability data sets (Lindenmayer et al. 1991, Mladenoff et al. 1995). The best-fit model was determined by applying the Akaike information criterion (Akaike 1974). To evaluate the predictive success of the selected model the Pearson chi-squared goodness-of-fit ($\chi^2$) and the coefficient of determination ($R^2$) were used to compare the validation data set to the predicted occurrence data (Paternoster et al. 1998, Guisan and Zimmermann 2000, Bowley 2008).

**Landscape Scale Predictions**

An area of 154 km$^2$ surrounding the survey area was used for landscape scale predictions of vernal pool abundance and maximum surface area. A slope layer was prepared for the landscape area as outlined above, with lake and wetland coverage removed from further analysis. Landscape scale terrestrial area ($R_j$; km$^2$) was calculated for each slope category ($j$), while landscape scale lake and wetland area (km$^2$) were calculated for all slopes together. Landscape scale vernal pool abundance ($A_j$; Equation 5) and maximum surface area ($M_j$; Equation 6) were predicted from terrestrial area ($R_j$).

$$A_j = R_j \times D_s \quad (5)$$

$$M_j = M \times A_j \quad (6)$$

The average maximum surface area ($M$) per pool was determined from field measurements at 37 pools in spring 2009. Surface area at each pool was calculated from measured North-South and East-West spring flooding extent, assuming an elliptical shape with axis lengths equal to extent measurements.
Results

The area surveyed was 2.8km², with slope ranging from 0° to 30.8°, and a mean slope of 6.3°. 89 vernal pools were identified during the field survey. One pool was excluded from further analysis due to its location in a cliff face. The 88 vernal pools analyzed occurred on slopes ranging from 0° to 6.8°, with a mean slope of 2.8°. Vernal pool density within the surveyed area was 31.4 pools km⁻². The average maximum surface area of pools (M) measured was 74.3 ± 16.7 m² (n=37, mean±SE).

The logistic model was the best-fit function to slope and vernal pool occurrence for the calibration data set (Figure 2, R²=0.980, p<0.0001). Predicted vernal pool occurrence values generated by the logistic model did not differ significantly from the observed validation data set (R²=0.976, p>0.05, X²-test). Within the logistic model, slope and probability of vernal pool occurrence were positively related below the inflection point, a slope of 2.0° (P=0.5), indicating areas of increasing vernal pool occurrence, negatively related from a slope of 2.0° to the upper slope limit of 7.0° (P=1.0), indicating areas of decreasing vernal pool occurrence, and unrelated above the upper slope limit, indicating vernal pool occurrence is rare above 7.0°. Vernal pool density within the surveyed area was 103.0 pools km⁻² where slope values were less than 2.0°, and 36.4 pools km⁻² between slopes of 2.0° and the upper slope limit of 7.0°.

Within the 154 km² surrounding the surveyed area, it is predicted that that 1645 pools, covering 0.12km², will occur below 2.0°, and 2127 pools, covering 0.16 km², will occur between 2.0° and 7.0° (Figure 3). The landscape-wide pool density, including all terrestrial and aquatic areas, is predicted to be 24.4 pools km⁻².

Discussion
A significant, predictive relationship exists between slope and vernal pool occurrence within the surveyed area, demonstrating that vernal pool distribution is clustered across the landscape (Figure 2). The logistic response found between slope and probability of occurrence indicates that the relationship varies by slope within the study area. Incremental probability increases with slope to a maximum at a slope of 2.0°, then decreases to a slope of 7.0°, above which vernal pool occurrence is rare. This relationship can reveal useful metrics for conservation planning involving vernal pools within the Frost Centre Area, as the inflection point indicates the slope of highest clustering and greatest conservation interest. Regions with slopes greater than 7.0° are unlikely to contain vernal pools and are of low conservation interest. The delineation of areas with slopes near the inflection point can be applied to identify the focal areas for field-based vernal pool surveys, reducing the need for photo-identification or complex multivariate modeling (Palik et al. 2003, Grant 2005, Lathrop et al. 2005). The upper slope limit can be applied to coarse-scale Climate Envelope Models to predict the distributions of species known to make use of vernal pools, such as the Wood Frog (Rana sylvatica) and many Salamanders (Abystoma spp.) (McKenney et al. 1998, Mackey and Lindenmayer 2001, Colburn 2004). Although this very simple model is strongly predictive for the study site, it does not necessarily represent a direct causal link between vernal pool occurrence and landscape slope, or explicitly account for more complex relationships between pool occurrence and additional landscape features, such as surficial geology or current land use. More research is needed to determine if the predictive capacity of this model holds when more complex landscape-level variables are accounted for (Calhoun et al. 2003, Palik et al. 2003, Grant 2005).
The tendency of vernal pools to occur in clusters on shallow slopes has been observed in previous studies (Creed et al. 2003, Grant 2005). However, distribution patterns found in different regions are difficult to compare directly, as the methods used to describe vernal pool occurrence patterns are varied. Further research is needed to validate the simple model derived in the present study at different scales and in regions with differing landscape features, including Minnesota, New Jersey, Maine, and Massachusetts (Calhoun et al. 2003, Palik et al. 2003, Grant 2005, Lathrop et al. 2005). If the model is applicable across varied scales and landscapes, then the slope at the inflection point and the upper slope limit may be simple and easily obtained metrics to compare the distribution of vernal pools among different geographical locations and landscapes.

The predicted landscape-level vernal pool density in this study, 24.4 pools km$^{-2}$, was much greater than that reported for other regions. In Minnesota, pool density averaged 8 pool/km$^{2}$ (Palik et al. 2003), and in Maine, density averaged 13.5 pools km$^{2}$ (Calhoun et al. 2003). This difference may derive in part from pool identification criteria. In the present study, a lower threshold for pool size was not used, whereas other studies applied minimum pool diameter limits ranging from 6m (Minnesota) to 38m (Massachusetts) (Palik et al. 2003, Grant 2005, Lathrop et al. 2005). If the higher density observed in this study resulted from inclusion of smaller pools, this would indicate that current vernal pool location methods with relatively high lower size thresholds may be excluding a large number of pools on the landscape, therefore underestimating vernal pool densities. Choosing areas with potentially high pool densities a priori for our field surveys could have inflated the predicted density. However, the predicted vernal pool
density was based on the entire landscape and the landscape would have needed to contain a larger proportion of low-slope areas than observed in previous studies to generate the higher overall pool density. The results may also simply reflect true differences in vernal pool density between regions, in which case our study site in Central Ontario may represent a fundamentally different pattern of vernal pool occurrence within the landscape than areas previously studied (Colburn 2004, Hortal et al. 2010).

Conclusions

This study presents a rapid, simple method for predicting the occurrence of a fine scale, point-based habitat feature (vernal pools) with widely available coarse scale data (slope). It demonstrates that a very simple model, developed with limited data, can be strongly predictive of vernal pool occurrence. The model developed may be utilized in its current form to refine spatial hypotheses represented by species area distribution models, or be expanded by additional research at different scales and in different regions. The simple logistic model used to relate vernal pool occurrence and slope accounted for a large proportion of variation, and was robust when validated with non-modeled data from the same study area. This predictive relationship within the study area suggests that the slope at the inflection point and upper slope limit are metrics of vernal pool distribution that may be applicable to coarse scale Climate Envelope Models or to fine scale field surveys (McKenney et al. 1998, Mackey and Lindenmayer 2001, Grant 2005, Lathrop et al. 2005). The relationship between vernal pool occurrence and slope demonstrated by the model is similar to that found in previous studies and may represent a useful method
to quantitatively compare vernal pool distribution between different regions, however validation of the model with external data is still required (Grant 2005).
Figure A1. Overview of the study area, including survey area boundaries (—we) and location of identified vernal pools (●), as well as lakes and wetlands. Vernal pools locations were identified in a field survey of 2.8 km2 within the Frost Centre Area, a mixed-wood upland forest in Central Ontario, Canada. Areas of high (■), low (■), and minimal (■) vernal pool density (Ds) were determined from field-identified vernal pool locations. Slopes were determined from a Digital Elevation Model (DEM, 1:20,000).
Figure A2. Cumulative probability of vernal pool occurrence (Oi) across increasing slope (i) for observations within calibration (●) and validation (○) data sets, as well as the predicted values from logistic regression fitted to the calibration data set (---) (R²=0.980, p<0.0001). Predicted vernal pool occurrence values did not differ significantly from the validation data occurrence values (R²=0.976, p>0.05, Χ²-test).

Verbal pool occurrence per slope category (Os) increased below the inflection point (s < 2.0o; Oi < 0.5), indicating areas of high vernal pool density, decreased between the inflection point and upper slope limit (2.0o ≤ s < 7.0o; Oi > 0.5), indicating areas of low vernal pool density, and approached zero above the upper slope limit (s ≥ 7.0o; Oi = 1.0), indicating minimal vernal pool density. Vernal pool locations were identified in a field survey of 2.8 km² within the Frost Centre Area, a mixed-wood upland forest in Central Ontario, Canada. Slopes were determined from a Digital Elevation Model (DEM, 1:20,000).
Figure A3. Area (km² and % total) of wetlands, lakes, and terrestrial (Rj) land cover, as well as predicted vernal pool abundance (Aj) and maximum surface area (Mj; km² and % total), within 154 km² of mixed-wood upland forest in Central Ontario, Canada.

Terrestrial and vernal pool areas were sub-divided by slope (j) to reflect areas of high (j < 2.0°; Ds = 103.0 pool/km²), low (2.0° ≤ j < 7.0°; Ds = 36.4 pool/km²), and minimal (j ≥ 7.0°; Ds = 0.0 pool/km²) vernal pool density (Ds). Vernal pools density and surface area were calculated from vernal pools identified in a field survey of 2.8 km² within the Frost Centre Area, a mixed-wood upland forest in Central Ontario, Canada. Slopes were determined from a Digital Elevation Model (DEM, 1:20,000) and water feature layers were obtained from the CanMap Atlas Bundle (Desktop Mapping Technologies (DMTI) Inc. 2009).
References


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