# Chapter 8
# Methods for Estimating Litter Decomposition

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**Abstract** Litterfall in terrestrial ecosystems represents the primary pathway for nutrient return to soil. Heterotrophic metabolism, facilitated through comminution by small insects and leaching during precipitation events, results in the release of plant litter carbon as $CO_2$ into the atmosphere. The balance between litter inputs and heterotrophic litter decomposition influences the amount of carbon stored in the forest floor. Periodic measurements of litterfall and litter decomposition with standard techniques will provide much needed information on carbon and nutrient cycling in forests. These available methods include mass balance, litterbags, tethered leaves, and the cohort layered screen. One must consider the strengths and limitations of each method as applicable to the goals of the study, and apply the most appropriate method, or combination thereof. For all methods, sufficient replication is required to accurately estimate stand level decomposition, and site selection for deployment should represent the various microsites likely to be encountered in the forest stand being examined.

**Keywords** Cohort screen, litterbags, litterfall, mass balance, tethered leaves

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## 8.1 Introduction

In terrestrial systems, plant litterfall is a primary pathway for the return of nutrients to the soil. Leaf tissue can account for 70% or more of aboveground litterfall in forests, with the remainder composed of stems, small twigs and reproductive structures (Robertson and Paul 1999). Litter decomposition proceeds by several mechanisms including heterotrophic utilization of organic compounds in litter, but also leaching during rain events and comminution by small insects which do not lead directly to $CO_2$ release to the atmosphere. The release of plant litter carbon (C) as $CO_2$ through heterotrophic decomposition by soil microorganisms can contribute 20% or more to soil surface $CO_2$ efflux, which is often referred to as soil respiration (Chapter 11, this volume, Raich and Nadelhoffer 1989). The balance between litter inputs and heterotrophic litter decomposition influences the amount of C stored in the forest floor; this is important because forest floor C can respond to disturbance over short time scales (e.g. Gaudinski et al. 2000). Further, nitrogen (N), phosphorus (P), and calcium (Ca) are released from plant litter during decomposition where they can become available for plant and microbial uptake. Given the important role of litter decomposition to C storage and tree nutrition, it is important to quantify litter decomposition rates for accurate characterization of forest carbon dynamics.

Litter decomposition rates are controlled by three main factors: temperature, moisture, and litter quality. Faunal community structure, especially the influence of earthworms, is increasingly being recognized as a possible fourth important factor (Bohlen et al. 1997, Dechaine et al. 2005). Where substrate is available, soil microbial activity increases exponentially with soil temperature, with microbial activity often doubling with a 10°C increase in temperature (Kirschbaum 1995). Microorganisms can also be limited by soil moisture. As temperatures increase, soil moisture assumes an increasingly important role for maintaining high rates of microbial activity (Peterjohn et al. 1994). As a result, rates of fresh litter decomposition increase with both increasing temperature and precipitation (Meentemeyer 1978).

This general pattern of decomposition can also be influenced by variability in litter quality. Quality refers to characteristics of the litter (chemistry, physical attributes, etc.) that influence the susceptibility of litter to decomposition. Litter containing high concentrations of labile compounds (e.g. sugars, amino acids) tends to decompose rapidly because these compounds can be readily metabolized by soil microorganisms or leached. For example, labile structural compounds such as cellulose are quickly cleaved by exoenzymes into sugar sub-units, which again are readily metabolized by microbial organisms. In contrast, recalcitrant structural compounds such as lignin and chitin are too large to pass through cell membranes, and are instead slowly processed by extracellular enzymes. Irregular chemical structure and complicated bonding make these compounds difficult for enzymes to attack, providing a slow release of N and P for continued microbial growth.

Three hypotheses are proposed to explain how initial litter quality influences litter decomposition and N release from decomposing litter (references as cited in Giardina et al. 2001). The first hypothesis suggests that litter decomposition and N

release are positively related to initial litter quality. In the early stages of decomposition the ratio of C:N may be the best predictor of mass loss and N release, with lignin content becoming increasingly important at later stages of litter decomposition. In the decay filter hypothesis, differences in initial litter quality (such as the ratio of lignin:N and lignin:cellulose) alter litter decomposition and release rates in the early stages of litter decomposition. As litter substrate quality decreases during decomposition, initial litter quality has a decreasing influence on late-stage decomposition rates. At this stage, litter decay rates are controlled instead by climate, soil texture, and exogenous sources of labile C and nutrients. The third hypothesis suggests that litter decomposition and rates of N release are negatively related to N-based estimates of initial litter quality. High N content may actually retard litter decomposition rates later in the decomposition process, particularly if lignin levels are also high. Regardless of the underlying mechanisms, periodic measurements of litterfall and litter decomposition with standard techniques will provide much needed information on C and nutrient cycling in forests.

## 8.2 Available Methods

### *8.2.1 Mass Balance*

Mass balance techniques are used to estimate litter decomposition for whole ecosystems, and are often employed when direct measurement is too cumbersome or expensive. When applied to aboveground litter decomposition, the mass balance approach suggests that annual litter decomposition should equal the annual input of fresh litter as long as the mass of detrital litter stored in the ecosystem remains constant (Olsen 1963, Schlesinger 1997). This approach assumes that a constant fraction, $k$, of the detrital litter mass decomposes, where

$$\text{litterfall} = k\ (\text{detrital litter mass}),\ \text{or}$$
$$\text{litterfall/detrital litter mass} = \text{k}.$$

For example, if the mass of the forest floor is 10 Mg C $ha^{-1}$ and annual litterfall is 1.0 Mg C $ha^{-1}$ $year^{-1}$, then litter decay rate would equal 0.1 $year^{-1}$. In forest ecosystems where decomposition rates are rapid and there is little surface litter accumulation, values for $k$ are greater than 1.0. Ecosystems with slow decomposition rates and surface litter accumulation, for comparison, have $k$ values that are less than 1.0.

From the equation, the method requires the collection of two variables: litterfall and detrital litter mass. Litterfall is measured using litter traps that are randomly spaced as appropriate throughout the study site (see Chapter 7, this volume, Bubb et al. 1998, Xu and Hirata 2002). Typically, litter trap openings are from 0.5 to 1 m across, and litter is contained within the trap by netting or a mesh screen. Traps are emptied at biweekly to monthly intervals, and collected materials can be sorted into

categories by litter type, species, and/or component, oven dried, and then weighed. Detrital litter mass, often called the forest floor and defined by USDA Soil Survey soil taxonomy as the Oi, Oe and Oa horizons, is estimated by removing the forest floor from a known area and, after drying the material, determining the dry weight. These quadrats are typically 1 x 1 $m^2$, though their dimension and frequency of measurement should be determined by the study site and objectives of the study. The forest floor is collected from inside the quadrat and sorted by component. The entire sample can be oven dried for dry weight determination, or wet weights can be measured and a subsample taken for dry weight determination. Because the Oa can contain up to 20% mineral mass, and soil can contaminate upper forest floor layers, forest floor mass should be corrected by sample combustion to determine the ash-free portion of the sample.

The mass balance approach can be used independently to estimate litter decomposition, or as a check on model predictions (Hedin 2000). It provides a robust estimate of litter decomposition at the stand level, though assumptions about steady-state stand conditions and constant forest floor decomposition dynamics complicates interpretation of calculated litter decay rates. Mass balance based estimates of litter decomposition are imprecise where short-term (e.g., annual) estimates are needed but forest floor mass is not in steady state. This method may not be appropriate in young stands where the forest floor is rapidly aggrading. In this case, the method would over-estimate decomposition rates. Because the method relies on native litterfall, this approach cannot be used to cleanly elucidate the role of other factors such as temperature and moisture as can common-litter litterbag experiments.

### *8.2.2 Litterbags*

The litterbag approach is widely used to study decomposition at the soil surface. Fresh leaf litter is enclosed in mesh bags, placed on the ground, and collected at periodic intervals for measurement of the mass remaining. A subset of the collected litter is oven dried to later establish wet to dry conversions for comparison. Mesh size is generally chosen to optimize access by all organisms to the litter while minimizing excessive particle loss, though mesh size can also be manipulated to exclude functional groups of litter decomposers. Very small mesh size will not only exclude certain organisms, but hinder particle loss to mineral soil as well. Fiberglass mesh has been recommended for light intensive sites where UV light will degrade nylon and other materials (Harmon and Lajtha 1999). Though 1–2 mm mesh is most common in litterbag studies (Robertson and Paul 1999), litterbag mesh size should be greater than 2 mm if a goal is to allow entry by macrofauna. Specific procedures for assessing the contributions of macroinvertebrates to decomposition can be found in Coleman et al. (1999).

Size and content of the litterbags is also an important component of litterbag studies. Overall bag size should be appropriate to the litter-specific ecosystem under consideration. While 20 x 20 cm bags are common (Robertson and Paul

1999), diverse plant communities or large leaf sizes may call for a larger litterbag. Litter should be freshly senesced. Litterbags are typically constructed with only one species, but when a more realistic experiment is desired, litterbags can be made with a proportionally representative mix of species litter and even small woody debris or reproductive structures.

The number of litterbags deployed at a site will depend on the variability of the site, the number of collections per year, and the number of years of the study. A forest with heterogeneous microclimate and stand characteristics will require a greater number of litterbags than an even-aged plantation to accurately calculate $k$. Typically, five or more replicate litterbags are collected at each sampling interval during the first year of the study, with two to four collections in subsequent years. This allows for a more robust characterization of the decay curve. Again, variability in stand micro-environment and overstory/understory diversity and associated litter quality should be considered before decisions about the number of required replicates is made.

Collected litterbags are oven dried in order to compare pre-and post-decomposition sample mass: separate samples may need to be freeze dried if substrate-specific chemistry will be analyzed. As with forest floor samples, mineral soil often contaminates litterbag samples, and should be corrected for by measuring the ash content of litter before and during decomposition. Litter decomposition rates are often estimated using a regression approach and the first order negative exponential decay equation, where the fraction of litter remaining after 1 year is given by:

$$X_t / X_o = e^{-kt} \tag{8.1}$$

where $X_t/X_o$ is the proportion of original mass remaining at time $t$, and $k$ is the decomposition rate constant. The decomposition rate constant, $k$, can be calculated by fitting the exponential decay model to a scatter plot of $t$ vs. $X_t/X_o$ (e.g. Harmon et al. 1999).

An alternate modified double exponential model that can provide a better fit for decomposition over the long term is given by:

$$Y = 100\exp(-kt^p) + \varepsilon \tag{8.2}$$

where Y is the original mass remaining at time $t$, $k$ is the decomposition rate constant, $p$ is a parameter allowing the mass loss rate to change with time, and $\varepsilon$ is the random component with a mean of 0 and variance of $\sigma^2$ (Kelly and Beauchamp 1987, Hanson et al. 2005). The decomposition rate constant $k$ and parameter $p$ are estimated from the data.

Litterbags have a few weaknesses and caveats regarding their use. Certain macroinvertebrates are excluded from the litterbags, lowering rates of litter comminution. Contamination by soils with high organic matter contents requires corrections. Care needs to be taken to ensure that the litterbag represents a realistic mixture of litter species and components, and that bag placement does not alter the

microclimate or decomposition conditions. Despite these limitations, litterbags represent a classic approach to estimating decomposition rates in the field, in particular because they can be used experimentally to quantify rates at various time scales and the contribution of different factors (e.g. temperature, moisture content). There have been many published litterbag studies, providing a rich database for comparison of results (see Vitousek et al. 1994).

### *8.2.3 Tethered Leaves*

The tethered leaf approach is similar to the litterbag approach, except that individual leaves are tied together in bundles rather than placed in litterbags. Either a single leaf, or groups of leaves, are tied together using nylon thread or monofilament fishing line. The line is tied to the leaf petiole for durability: the line is usually anchored to both a reference point for collection, and an identifying tag.

A "wheel spoke" approach modeled after Vitousek et al. (1994) is often employed in terrestrial studies. A representative group of individual senescent leaves are air-dried in the laboratory and tied by their petioles to a single line. One end of the line is tied to an identifying tag, and the other end to a flagged washer. Several groups of strings are tied to each washer in this manner: the washer provides the hub, and individual lines the spokes. At each collection interval, one or more lines are snipped from the hub, and measured for decomposition. Subsamples of each line of senescent leaves are then oven dried to determine air-dry/oven-dry ratios. While leaves on a given line usually are weighed individually, mass loss and elemental concentrations are determined for the group to account for any loss of whole leaves. The same care needs to be taken as outlined in the litterbag approach, where leaf litter composition is reflective of the stand under study.

Tethered leaf studies are most useful in studying the early stages of decomposition, thus length of study is not as important as with litterbag approaches. As leaves begin to fragment (a common occurrence early in the decomposition process) this technique will over-estimate decomposition rates relative to the litterbag approach: bag mesh will retain large leaf fragments that would otherwise be lost with the tethered leaf method. Because of the large influence of comminution on estimated decomposition rates for thin or easily fragmented leaves, this method may best yield insights into leaf litter quality for thicker leaves. Studies have shown that small, litter-feeding invertebrates have ready access to litter in litterbags with mesh sizes as low as 1.5 mm (Scowcroft et al. 2000). However, the tethered leaf approach allows for leaf consumption by macroinvertebrates such as crabs and snails, whose access would otherwise be restricted by mesh bags (McKee and Faulkner 2000). Litter is also in direct contact with forest floor, removing methodological artifacts such as changes in forest floor temperature and moisture status.

### *8.2.4 Cohort Layered Screen*

A fourth approach to estimating aboveground leaf litter decomposition is the cohort layered window screen method, or litter sandwich method. With this method, layers of mesh screen are used to separate successive layers of litter on the forest floor; leaf litter then decomposes *in situ*.

The cohort layered screen method is applied to long-term decomposition studies, typically three or more years in duration, and is described in detail elsewhere (Binkley 2002). Following major annual litterfall, a layer of window screen is placed over the forest floor. Typically, 1 x 1 m fiberglass or aluminum window screening with a mesh size of 2–3 mm is used. The screen size will depend on the size of the stand sampled, and mesh size will vary with the specific ecosystem under study (see discussion of mesh size under litterbags section). Fiberglass screen is recommended over aluminum if any chemical or constituent properties will be analyzed as well. Following each subsequent annual litterfall for the duration of study, another layer of screen is placed directly over the screen from the previous year. After a given sampling period, subsamples of the original screen can be cut from the original to obtain data while allowing the experiment to continue. Subsamples are collected, weighed, and oven-dried. These are compared with stand level estimates of litterfall for the year in question.

While the litterbag and tethered leaf methods raise concerns about representative leaf quality, the cohort method applies a realistic input of litter species and components, providing the entire litter input for decomposition. It is relatively easy to monitor, as monetary and material resources for preparation and collection are both low, and has been found to represent litter dynamics in the forest floor better than litterbag studies. Such litter sandwiches integrate a large portion of the forest floor, especially for long-term studies. However, the cohort layered screen method also excludes certain macrofauna that are blocked from access to leaf litter by the mesh screen, and can alter the forest floor microclimate.

## 8.3 Summary

All methods for quantifying litter decomposition suffer from the same inability to separate decomposition losses from leaching and comminution. The distinction is important, but rarely addressed, because the former results in C return to the atmosphere as $CO_2$ while the latter two processes bring detritus into the soil food web where C may or may not return to the atmosphere. Decomposition constants derived from the litterbag and cohort approaches are fundamentally difficult to scale to the stand because the micro-environment created by both methods and exclusion of organisms will create artifacts. The litterbag approach has the additional problem of accurately representing the forest floor matrix in each litterbag. Comminution losses out of litterbags or through window screen of the cohort approach are of smaller concern than with the tethered litter approach where a

break at the petiole is interpreted as decomposition. For all methods, sufficient replication is required to accurately estimate stand level decomposition, and site selection for deployment should represent the various microsites likely to be encountered in the forest stand being examined. This becomes exceedingly difficult to do in diverse stands with complex microtopography. There is also the question of how to capture the decomposition of older material, especially the more decomposed Oa/Oe horizon materials.

For these reasons, it is difficult to impose a "one size fits all" strategy for estimating litter decomposition. One must fully consider the strengths and limitations of each method as it applies to the goals of the study. Since litterfall and forest floor mass will be collected at landscape-scale monitoring sites, one might assume that the mass balance approach would be an easily applicable model, incurring little additional expense. However, as noted earlier, this approach can be imprecise at a scale of annual resolution. If higher resolution is required (e.g., decomposition of branches versus leaves), or forest floor mass is dynamic (e.g., young stands following fire), the cohort layered screen could be used at reasonably small expense. Exclusion of macrofauna, though, could lead to an underestimation of decomposition rates. A realistic approach may be to pair a combination of these techniques as required by various characteristics of each site.

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