CHAPTER 5

LEACHING

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1. INTRODUCTION

The decomposition of autumn-shed leaves has traditionally been subdivided into three more or less distinct phases: leaching, microbial colonization and invertebrate feeding (Petersen & Cummins 1974, Gessner et al. 1999). Leaching is defined as the abiotic removal of soluble substances, among them phenolics, carbohydrates and amino acids (for analyses of these compounds, see Chapters 10, 11 and 14). It is largely completed within the first 24—48 h after immersion in water, and results in a loss of up to 30% of the original mass, depending on leaf species. Gessner & Schwoerbel (1989) showed that no such rapid leaching loss can be observed when fresh, rather than pre-dried, alder and willow leaves are used. Fungal colonization proceeded more slowly on fresh than on pre-dried alder and willow leaves (Bärlocher 1991, Chergui & Pattee 1992), dynamics of chemical leaf constituents differed between fresh and pre-dried leaves during subsequent decomposition (Gessner 1991), but no effects on invertebrate colonization have been observed (Chergui & Pattee 1993, Gessner & Dobson 1993). In a survey of 27 leaf species, drying significantly changed the magnitude of leaching in a majority of cases (Taylor & Bärlocher 1996), although the direction of change was variable among species with drying actually decreasing leaching in several cases. Some representative data are listed in Table 5.1.

Changes in types and amounts of compounds retained by leaves may affect their breakdown rate by selectively stimulating or inhibiting colonization by aquatic microorganisms (Bengtsson 1983, 1992) and by modifying palatability to leaf-eating invertebrates (review in Bärlocher 1997). In addition, they will influence the dynamics of the dissolved organic matter pool in the water column, its flocculation into solid particles (Bärlocher et al. 1989), and its entrapment and processing at liquid-solid interfaces (Armstrong & Bärlocher 1989a,b, Meyer et al. 1998, Allan 1995).

M.A.S. Graça, F. Bärlocher & M.O. Gessner (eds.), Methods to Study Litter Decomposition: A Practical Guide, 33 – 36.

Table 5.1. Percentages of mass losses of fresh and dried leaves over 48 h in distilled water. Mean±SD. <, loss significantly greater in dried leaves; =, no significant difference; >, loss significantly greater from fresh leaves. Data from Taylor & Bärlocher (1996).

	Leaf mass loss (% dry mass)		
Leaf species	*Fresh*		*Dried*
Acer saccharum	15.2±7.9	<	21.4±7.6
A. negundo	14.7±3.3	<	30.5±2.1
A. circinatum	6.3±5.2	<	23.7±1.5
A. rubrum	16.6±9.2	=	24.5±8.2
Fagus grandifolia	5.1±7.0	=	7.4±6.6
A. macrophyllum	10.2±6.7	>	5.9±0.9
Betula papyrifera	15.1±2.4	>	11.7±1.3

In some areas, the yearly leaf fall may overlap with the first night frosts. Freezing living or senescent leaves can have a similar effect as drying them: it may damage cell membranes, which generally accelerates leaching (Bärlocher 1992). In other areas, leaf senescence may coincide with hot, dry weather, and leaves may dry on the tree.

The method described here allows assessing how drying leaves influences leaching. Freshly collected, non-dried leaves (fresh leaves) and leaves that are dried after collection (dried leaves) are exposed in fine-mesh bags (to prevent access by macroinvertebrates) in a stream. After four days, the remaining mass is measured. During this early period of decomposition, leaching generally predominates. If drying significantly increases leaching, we expect higher losses in dried leaves. If desired, identically treated leaves can be examined for colonization by aquatic hyphomycetes. To study the temporal course of leaching losses in greater detail, leaf bags should be prepared to allow daily samples for an extended period of seven days. Or, leaves can be submerged in distilled or stream water in the laboratory, and daily samples can be taken (Gessner & Schwoerbel 1989, Taylor & Bärlocher 1996).

2. EQUIPMENT, CHEMICALS AND SOLUTIONS

2.1. Equipment and Material

- Oven (40—50 °C)
- Leaves of *Alnus glutinosa* or other species
- Litter bags (10 x 10 cm, mesh size 0.5 or 1 mm)
- Plastic labels
- Balance (±1 mg)

3. EXPERIMENTAL PROCEDURES

3.1. Sample Preparation

1. Dry leaves: collect leaves from a single tree by gently shaking branches and collecting fallen leaves. Dry for 2 days at 40—50 °C to constant mass. Randomly select 2— 3 leaves and weigh to the nearest mg. Moisten leaves to avoid breakage by placing the leaves in a small tray and spraying them with water (avoid highly chlorinated tap water), and place them in a litter bag (see Chapter 6). Label the bag. Prepare a total of 20 bags.
2. Fresh leaves: harvest leaves from the same tree. Return them to laboratory in a cool, closed container. Randomly select 2—3 leaves, weigh them, and place them in a litter bag. Label the bag. Prepare a total of 20 bags. To determine wet mass/dry mass ratio of fresh leaves, individually weigh 20 fresh leaves, dry them, and weigh them again.

3.2. Experiment

1. Expose all bags in a stream.
2. Recover all bags after 4 days.
3. Rinse leaves under running tap water, dry at 40—50 °C to constant mass, and weigh them.
4. Express mass loss as percentage of original leaf mass.

3.3. Statistical Analysis

Mass losses of fresh and dried leaves can be compared with a *t*-test or a permutation test (see Chapter 43). Since some values are likely to be below 20%, normal distribution cannot be assumed, and arcsine transformation of proportion p is advisable before applying a standard *t*-test ($p' = \arcsin \sqrt{p}$).

For the permutation test, we assume that the values for fresh and dried leaves belong to the same population (H_0, null hypothesis). We therefore pool all values. Next, we randomly divide the 40 values into two groups of 20. We determine the difference between mean mass losses of the two groups. We do this 10000 times and plot the distribution of the differences. Next, we determine the actual difference between the original data from fresh and dried leaves. How “extreme” is it? If it is at least as extreme as 5% of the population of differences based on the permutated data (this corresponds to $p \leq 0.05$), we reject the null hypothesis.

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