

PERSPECTIVES

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A comment on the use of exponential decay models to test nonadditive processing hypotheses in multispecies mixtures of litter

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Abstract. Many studies testing hypotheses about the effects of leaf litter diversity on litter processing rates contain a systematic bias that stems from mathematically incorrect assumptions. Assuming that the mean of single-species processing rates adequately describes noninteracting mixed-species rates will consistently underestimate or miss positive interactions between species. Further, the slope of log-transformed mass loss of litter mixtures vs time is affected by the duration of the experiment. Simple solutions to these errors include separating species from litter mixtures after incubation, or comparing masses remaining rather than comparing slopes.

Key words: litter processing, litter diversity, nonadditive processing.

Decomposition of litter is an important ecosystem process that provides energy to and recycles nutrients in heterotrophic communities. In seasonal forested ecosystems, periodic synchronous abscission of leaves delivers a significant, and sometimes overwhelming, pulse of litter to the forest floor and the channels of streams draining these forests (Fisher and Likens 1973). These leaves are colonized by bacteria and fungi, and this leaf–microflora complex is consumed by a variety of detritivores (Arsuffi and Suberkropp 1984). The rate at which leaves are processed is measured as mass loss with time and is affected by physical characteristics of the system and by litter species characteristics such as C:N, lignin:N, and a variety of other measures of leaf quality (Melillo et al. 1982, Ostrofsky 1997).

Processing rate coefficients, k , are calculated as the slope of the log-transformed regression of litter mass remaining vs time (Petersen and Cummins 1974). Some researchers (e.g., Wieder and Lang 1982) have argued against the use of simple exponential decay

equations, but their application is nearly universal, and if leaves are preleached for 24 h or if regression analyses do not include the initial (time = 0) mass, exponential decay equations probably best describe mass loss. Both approaches minimize the effect of rapidly solubilized leaf constituents. Most of what we know about leaf processing comes from studies of mass changes in single-species leaf packs. However, monospecific litter assemblages are probably rare in forests and rarer still in stream systems where flow integrates litter inputs longitudinally.

The possibility exists that litter diversity affects decomposition through species interactions, and several hypotheses might be considered in systems that receive inputs of mixed species of autumn-shed leaves. First, a slowly processed species might lose mass faster as a consequence of being adjacent to a more rapidly decomposing species—presumably its mass loss would be facilitated by the proximity to greater nutrient concentrations that enable earlier and more rapid colonization of the slowly processed species by decomposing microflora than would occur in a single-

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species leaf pack (Chadwick and Huryn 2003). Second, a rapidly processed species might lose mass more slowly as a consequence of being adjacent to a slowly processed species—presumably its mass loss would be inhibited by the proximity to higher concentrations of water-soluble defensive or other inhibitory compounds (e.g., tannins and other phenolics) in the slowly processed species (Harrison 1971). Third, the structural stability imparted to a mixed-species leaf pack by slowly processed species might either provide a more persistent habitat for decomposing organisms, thereby accelerating the rate of processing, or might protect otherwise rapidly processed species from the weakening effects of abrasion and leaching, thereby slowing the rate of processing. Last, because none of these effects is necessarily mutually exclusive, some or all might be operating in concert so that facilitation and inhibition occur simultaneously, and observed mass changes in mixed-litter packs represent the net effect of multiple processes.

Several experiments have been designed to determine if multispecies litter has a nonadditive processing rate; that is, if the processing rate of mixed-species litter differs from what might be expected from the processing rates of the component species considered separately. Gartner and Cardon (2004) recently compiled the results of 22 terrestrial (and 1 aquatic) studies where mass losses were monitored in single- and mixed-species leaf packs. Similar studies in aquatic systems are much less common (Table 1). Nevertheless, results from both environments support the idiosyncratic hypothesis (Lawton 1994) that litter diversity can affect processing, but with unpredictable magnitude and direction.

Approaches to Predicting Mass Loss in Mixed-Species Litter

In many studies, positive interaction strength may have been underestimated or may have escaped detection entirely because of a widespread methodological error. A common approach when seeking nonadditive effects has been to compare the processing rate or mass loss from mixed-litter assemblages to the rate or mass loss anticipated from knowledge of rates or losses of component species individually. These comparisons can take 2 forms. First, *k* values of individual species are averaged as an estimate of mixed-species processing rates, and this average rate is used to calculate the expected mass remaining in mixed-species litter after some period of time (Taylor et al. 1989, Kaneko and Salamanca 1999, Swan and Palmer 2004, 2006, Schweitzer et al. 2005, LeRoy and Marks 2006). Second, log-transformed mass changes

TABLE 1. Studies of mixed-species mass loss in aquatic systems.

| Reference | Species | Methods/duration | Results |
|------------------------|---|--|--|
| Leff and McArthur 1989 | Red maple (<i>Acer rubrum</i>) and cypress (<i>Taxodium distichum</i>) | 4-mm-mesh bags/14 wk | Cypress had no effect on red maple processing rate |
| McArthur et al. 1994 | Water oak (<i>Quercus nigra</i>) and sweetgum (<i>Liquidambar styraciflua</i>) | Tethered leaf packs, single and mixed species/91 d | Sweetgum had no effect on oak; oak slowed sweetgum decomposition |
| Bailey et al. 2003 | Swamp paperbark (<i>Melaleuca ericifolia</i>), common reed (<i>Phragmites australis</i>), and water ribbon (<i>Triglochin procerum</i>) | 0.5-mm-mesh bags (species double bagged)/15–86 d | Paperbark had marginal effect on water ribbon, no effect on common reed |
| Swan and Palmer 2004 | Silver maple (<i>A. saccharinum</i>), box elder (<i>A. negundo</i>), sycamore (<i>Platanus occidentalis</i>), black walnut (<i>Juglans nigra</i>), slippery elm (<i>Ulmus rubra</i>), and black willow (<i>Salix nigra</i>) | 8 × 3-mm-mesh bags with 1–5 species/84–87 d | Most multispecies treatments lost mass more slowly than predicted in summer; no difference in autumn |
| LeRoy and Marks 2006 | Velvet ash (<i>Fraxinus velutina</i>), Arizona alder (<i>Alnus oblongifolia</i>), Fremont cottonwood (<i>Populus fremontii</i>), Arizona sycamore (<i>P. wrightii</i>), and Gambel oak (<i>Q. gambelii</i>) | 6.4-mm-mesh bags with 1, 3, 5 species per bag/83 d | 5-species mix lost mass faster than expected at only 1 of 3 sites; 1 of three 3-species mixes lost mass faster than expected |
| Swan and Palmer 2006 | Boxelder (<i>A. negundo</i>), American sycamore (<i>P. occidentalis</i>), black willow (<i>S. nigra</i>), black walnut (<i>J. nigra</i>), and slippery elm (<i>U. rubra</i>) | 1- and 4-species mesh bags/67 d | 4-species mix without boxelder lost mass more slowly than expected; 4-species mix without elm lost mass faster than expected |

from single- and mixed-species packs are plotted as a function of time, and the resulting slopes are compared using *t*-tests or analyses of covariance (Taylor et al. 1989, Blair et al. 1990, Scowcroft 1997, Ashton et al. 1999). Each of these approaches is straightforward and convenient, but both follow from a mathematically incorrect assumption.

A more appropriate approach is to assume that mass remaining is a species-specific exponential function of time. A mixed 2-species pack would lose mass according to a double exponential function:

$$W_{(a+b)t} = W_a e^{-k_a t} + W_b e^{-k_b t} \quad [1]$$

where $W_{(a+b)t}$ is the mixed-pack mass remaining at time t ; W_a and W_b are the initial masses of species a and b , respectively; and k_a and k_b are the processing rate coefficients for species a and b alone, respectively. Three- and four-species packs would lose mass with similar triple and quadruple exponential functions, etc. I will limit my comments to double exponential functions because most studies cited by Gartner and Cardon (2004) and those in Table 1 test nonadditive hypotheses using 2-species packs.

The litter mass remaining value predicted from a double exponential function can differ markedly from the mass predicted from a single exponential function. As a simple example, consider hickory (*Carya glabra*) and beech (*Fagus grandifolia*), both widely distributed species in the deciduous forests of eastern North America. The mean values of k tabulated by Webster and Benfield (1986) for these species differ by more than an order of magnitude (0.0158/d and 0.0008/d, respectively). What might be the processing rate of leaf packs that consist of equal masses of these 2 species? Using the above processing rates for the individual species and the double exponential equation (equation 1) and assuming an initial mixed-pack dry mass of 10 g (5 g of each of the 2 species), the anticipated mass remaining after 100 d would be 5.65 g, and after 150 d, it would be 4.90 g.

In comparison, the 1st approach (described above) would use the mean processing rate of the 2 species and predict remaining masses of only 4.36 g after 100 d and 2.88 g after 150 d. The different outcomes of the 2 models are illustrated for this example in Fig. 1. Note that the mean of the 2 single-species rates always predicts lower litter mass remaining than the double exponential model does. Hence, if this average processing rate is the basis for the anticipated outcome under the assumption of no species interaction, then experiments will commonly underestimate or miss positive interactions between species.

The 2nd approach (described above) would eliminate calculations with either model. In this case, the log-

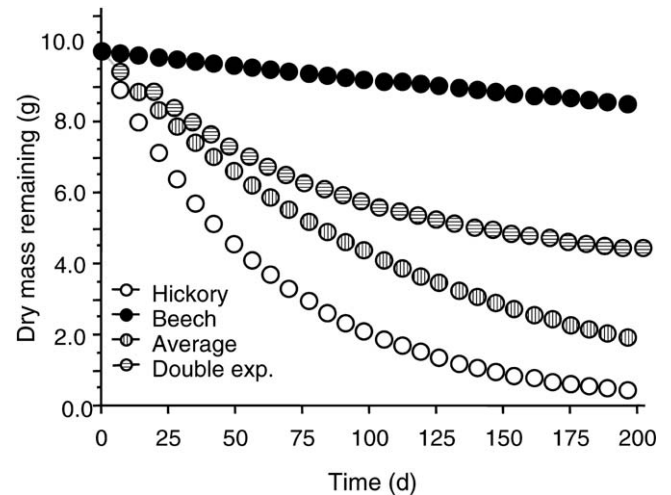


FIG. 1. Hypothetical example to illustrate the effect of model choice on anticipated processing. Mass remaining in monospecific packs of both hickory and beech are calculated using mean decay rate coefficients taken from Webster and Benfield (1986). The mass remaining in mixed-species packs is calculated using the mean of the 2 species-specific coefficients and the double exponential (exp.) model. All simulations are based on initial litter dry mass of 10 g (5 g of each species in mixed treatments).

transformed mass remaining in mixed-species packs would be plotted as a function of time, and the slopes of single-species packs would be compared with slopes of mixed-species packs using *t*-tests or analysis of covariance. The problem again is the assumption that mixed-litter processing can be explained with a single, rather than a double, exponential equation. Mass remaining in a double exponential equation is *not* linearized by a simple natural log transformation (as has been assumed), and the slope (k) of the best-fit line of the natural log-transformed data regressed against time is disproportionately affected by the fast species if experiments are of short duration, with increasingly disproportionate influence from the slow species as incubation times become longer. Considering the hickory and beech example once again, if the experiment had lasted only 6 wk, with weekly samples taken, the calculated slope ($\log[\text{mass remaining}]$ vs time) in the mixed-species treatment would have been 0.0071/d. In longer experiments, the influence of the slow species would increase. Had the same experiment lasted 25 wk, the calculated slope would have been 0.0042/d. In general, not enough data are collected across the large number of time intervals necessary to use alternate curve-fitting techniques (Riggs 1963), but in any case, comparison of mass-remaining curves with varying slopes to those with constant slope is problematical.

Obviously, if the processing rates of 2 species are similar, the error created by averaging the 2 rather than using a double exponential function is small and probably not detectable given the variability in leaf-pack replicates typically seen. However, most experiments designed to search for nonadditive effects select species with anticipated large differences in processing rates. In any case, using inappropriate mathematics has no justification.

Alternative Approaches

These analytical problems can be avoided in simple ways. One approach is to separate the 2 species during processing of mixed-species treatment bags after incubation and calculate remaining masses of each (e.g., McArthur et al. 1994, Conn and Dighton 2000). In this case, the slopes of the log-transformed mass remaining vs time can be compared in single- and mixed-species treatments. It becomes increasingly difficult to recognize and separate species after prolonged exposure, but most tests of nonadditive processing hypotheses pair a rapidly processed species with a slowly processed one that remains identifiable for a long time. In the beech example used above, almost 90% of the original mass would remain after 19 wk of incubation, so it would be relatively easy to identify and separate beech leaves from species mixtures. Recognition can be further facilitated by systematically alternating leaves of 2 species in the leaf packs or mesh bags. This approach has the added advantage of making it possible to examine the effects of litter diversity on the processing rates of each of the component species, rather than measuring the net effect on the 2 species combined.

Alternatively, anticipated mass remaining in mixed packs could be estimated as the sum of the masses predicted based on single-species processing rates (Wardle et al. 1997, Bardgett and Shine 1999). For example, if single-species packs result in processing rates of 0.0158/d and 0.0008/d for hickory and beech, respectively, then the null hypothesis (no interaction) would predict remaining masses of 8.10, 6.84, and 6.01 g after 4, 6, and 8 wk, respectively, in mixed packs starting with 5 g of each of the 2 species. Differences could be detected through matched (by time) paired *t*-tests. However, with increasing time, the mixed-pack mass remaining would converge on the mass predicted by the null hypothesis as the more rapidly processed species contributed less and less to the total mixed-pack mass.

It is not clear how often positive interactions between decomposing species may have been missed or underestimated, but the assumption that mixed-species

processing can be described by a single exponential decay function introduces a consistent bias against finding these interactions. The examples herein illustrate more scientifically sound approaches for addressing nonadditive litter processing hypotheses.

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