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REGULAR ARTICLE

Assessing interactive responses in litter decomposition in mixed species litter

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Abstract The aim of this research is to propose an improved method to partition single species contributions to decomposition in mixed species litters and to detect additive or non-additive responses in litter decomposition. Using simulated data, we demonstrate that additive responses can arise from multiple conditions, including no changes in litter decomposition rates of both species in the mixtures, or an enhanced decomposition of one species and a reduced decomposition of another. Antagonistic responses can be caused by reduced decomposition of only one species, or of both species. Without partitioning of the contributions of single species proposed here, it is difficult to distinguish the different causes of the overall responses. Our analyses provide a better understanding of litter decomposition in mixtures and have significant implications for modeling litter decomposition.

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Introduction

Litter decomposition plays an important role in carbon and nutrient cycling in terrestrial and aquatic ecosystems (Schlesinger 1997; Norby and Jackson 2000; Liski et al. 2003; Hui and Luo 2004; Swan and Palmer 2004; Kurz-Besson et al. 2006; LeRoy and Marks 2006; Fang et al. 2007; Taylor et al. 2007). It has been studied for decades, with many studies focusing on single species litter decomposition. Recent results show that single species litter decomposition may not represent natural ecosystems where multiple species decompose together (King et al. 2002; Hoorens et al. 2003; Wardle et al. 2003; Hättenschwiler and Gasser 2005; Schweitzer et al. 2005; Moore and Fairweather 2006; Chapman and Koch 2007; LeRoy et al. 2007). By reviewing about 30 papers on litter decomposition in both mixed species and single species litter, Gartner and Cardon (2004) found that 67% of all mixtures tested (108 out of 162) exhibited non-additive mass loss (i.e., different responses of decomposition in the mixtures compared to the expected responses estimated from single species litter). Thus, evaluating the overall response in mixed species litters and the contributions of single species is critical to our understanding of litter decomposition and for modeling carbon and nutrient processes in terrestrial ecosystems.

To detect the interactive effects of plant species in litter mixtures, most studies incubate leaf litter in litter bags of single species and mixed litter of different species over a certain period of time, then compare the observed mass remaining in mixed species litter with the expected single species values (Wardle et al. 2003; Swan and Palmer 2004; Quested et al. 2005; LeRoy and Marks 2006; Lecerf et al. 2007). Expected mass remaining in the mixtures is mostly calculated as the mean of mass remaining in single species litter decomposing alone by assuming that there is no interaction among species. Analysis of variance (ANOVA) or *t* test is often used to test the significance of the differences between the observed and expected mass remaining (Gartner and Cardon 2004; Ganjegunte et al. 2005; Quested et al. 2005). If the observed mass remaining in the litter mixtures matches the expected value, it is called an additive response. Otherwise, a non-additive response is detected. If the overall decomposition in the mixtures is enhanced relative to what is expected based on single-species dynamics, it is a synergistic response. Otherwise, it is an antagonistic response. However, this ANOVA method requires independent, random sampling, normality of distributions and homogeneity of variance in the error term assumption which may be difficult to satisfy (Gartner and Cardon 2004). ANOVA can also be problematic when the interaction of the main effect and time is significant (i.e., difference between observed and expected values varies with time; Gartner and Cardon 2004; Ostrofsky 2007).

Litter decomposition rate has long been estimated using an exponential decay function (Olson 1963). Similar to the comparison of mass remaining, interaction of species can be detected by comparing the observed and expected litter decomposition rates in the mixtures (McArthur et al. 1994; Swan and Palmer 2004; Schweitzer et al. 2005; LeRoy and Marks 2006; Dearden et al. 2006; Taylor et al. 2007; Kominoski et al. 2007). However, the calculation of the expected decomposition rate using the arithmetic mean provides a higher value than the true expected decomposition rate (see "Materials and methods"). Thus, an additive response might be detected as a non-additive, antagonistic response. Recently, Ostrofsky (2007) nicely demonstrated the problem of simple arithmetic average of two decomposition rates, and provided a method to accurately estimate the expected mass remaining in the mixtures. But all the methods proposed so far can only detect an overall additive or non-additive response in mixed species litter. The contributions of single species in mixed species litters cannot be separated. Most problematically, some interactive effects (e.g, decomposition of one species is enhanced while another is reduced in the mixtures) can only be detected as an overall additive response. We suggest an improved method to estimate the expected decomposition rate in mixed species litter and develop a new approach to separate the contributions of single species in the mixtures.

Materials and methods

Detecting additive or non-additive responses in mixed species litter

For simplicity, suppose we have two species, A and B. We incubate litter of species A and B alone and in combination. At the beginning of the experiment, all litter bags have the same amount of dry mass. For the mixed species litter AB, equal proportions of species A and B are placed in the bags. The litter bags are incubated in the field for a series of time periods. At each harvest, litter mass remaining in single species litter bags and in mixed species litter bags is measured. Let $Y_{\mathrm{A}t}$, $Y_{\mathrm{B}t}$, and $Y_{\mathrm{AB}t}$ represent the fraction of mass remaining at time t for species A, B and mixed species litter AB, respectively. If the decomposition of leaf litter follows an exponential decay function, we can fit the observed data with the following equations (Olson 1963)

$$\widehat{Y}_{\mathrm{A}t} = e^{-k_{\mathrm{A}}t}, \tag{1}$$

$$\widehat{Y}_{\mathrm{B}t} = e^{-k_{\mathrm{B}}t}, \tag{2}$$

and

$$\widehat{Y}_{\mathrm{AB}t} = e^{-k_{\mathrm{AB}}t}, \tag{3}$$

where k_{A}, k_{B}, and k_{AB} are litter decomposition rates for species A, B, and mixture AB, respectively, which are to be estimated; t is time.

The expected mass remaining in mixed species litter AB can be calculated as the means of mass remaining for the two single species A and B under the assumption that there is no interaction between these two species. Thus, the expected fraction of mass remaining in mixed species litter for an equal mixture A and B is

$$\overline{Y}_{ABt} = \frac{1}{2} Y_{At} + \frac{1}{2} Y_{Bt}. \quad (4)$$

Based on $\overline{Y}_{ABt}$ and time t, the expected decomposition rate in mixed litter can be estimated by the exponential decay function

$$\widehat{\overline{Y}}_{ABt} = e^{-\overline{k}_{AB}t}. \quad (5)$$

where $\overline{k}_{AB}$ is the expected decomposition rate in mixed species litter. Comparison of k_{AB} with $\overline{k}_{AB}$ can be used to determine whether an additive or non-additive responses occur. If the 95% confidence interval of k_{AB} does not include $\overline{k}_{AB}$, we consider that there is a significant difference between these two, and accept that a non-additive response exists.

Due to the nonlinear nature of the exponential decay function, the expected decomposition rate in mixed species litter estimated here $\left(\overline{k}_{AB}\right)$ is usually smaller than the arithmetic mean of the single species decomposition rates themselves $\left(\overline{k}_{AB} < (k_A + k_B)/2\right)$ used in many studies (McArthur et al. 1994; Swan and Palmer 2004; Schweitzer et al. 2005; Dearden et al. 2006; LeRoy and Marks 2006; Taylor et al. 2007; Kominoski et al. 2007). Thus, using the arithmetic mean of decomposition rates of single species would overestimate the expected decomposition rate, and may erroneously detect an additive response as a non-additive, antagonistic response.

Partitioning single species contributions in mixed species litters

Similar to the example above, suppose we have litter of two species, A and B, and one mixed species litter, AB, incubated and measured for a period of time. Litter decomposition rates for species A and B alone (i.e., k_A and k_B) are estimated using Eqs. 1 and 2. For the mixed species litter Y_{ABt}, we fit a double exponential decay curve

$$\widehat{Y}_{ABt} = \frac{1}{2} e^{-f_A k_A t} + \frac{1}{2} e^{-f_B k_B t} \quad (6)$$

where f_A and f_B are enhancement factors for species A and B that represent the relative change of litter decomposition rates in mixed species litter compared to that in single species litter; k_A and k_B are two constants representing litter decomposition rates for species A and B estimated using the single species measurements above (Eqs. 1 and 2). In Eq. 6, only the enhancement factors f_A and f_B are unknown parameters and need to be estimated. Since this equation can not be transformed into linear regression equation and there is no analytical solution for this equation, nonlinear regression methods should be used to estimate the parameters (Hui and Jackson 2007; SAS Institute Inc. 2008). Usually numerical optimization algorithms are required to determine the best-fitting parameters. Ordinary least squares approach can be used to minimize the sum of squared deviations $\left(SS_e = \sum (Y_{AB} - \widehat{Y}_{AB})^2\right.$. Several algorithms such as Gauss–Newton method can be used to search parameters f_A and f_B until SS_e is minimized (Glantz and Slinker 2001; Nerlove 2005). Asymptotic standard errors of parameters A and B can then be calculated as $s_f = \sqrt{SS_e/(n-2)\mathbf{H}^{-1}}$, where n is the number of mass remaining measurements, $\mathbf{H}$ is Hessian matrix containing the second derivatives of parameters. The test of significant difference of enhancement factor for species A and B with 1 can be conducted using the t test (Hui and Jiang 1996):

$$t_A = \frac{f_A - 1}{s_{f_A}} \quad (7)$$

and

$$t_B = \frac{f_B - 1}{s_{f_B}} \quad (8)$$

If f_A or f_B is not significantly different from 1, then no change in decomposition rate occurs in the mixtures for species A or B; if f_A or f_B is significantly larger than 1, then the litter decomposition rate is enhanced in the mixtures; if f_A or f_B is significantly less than 1, then the decomposition rate is reduced in the mixtures.

The changes in litter decomposition rate of a single species would be reflected in the overall responses in the mixtures and could be helpful in explaining the interactive response in the mixed species litter. If both f_A and f_B are not significantly different from 1, then the overall response in the mixture would be an additive response; if f_A>1 and f_B>1, then synergistic response in the mixture would occur; if f_A<1 and f_B<1, an antagonistic response in the mixture would be detected.

However, if $f_A>1$ and $f_B<1$, or $f_A<1$ and $f_B>1$, both additive and non-additive responses (including synergistic and antagonistic responses) could occur. Without partitioning the single species contributions proposed here, it is impossible to distinguish a true additive response (i.e., no change in decomposition rates of both species) from an interactive response where enhanced litter decomposition of one species cancels out a reduced litter decomposition of another.

The framework for two species developed here could be extended to multiple species and/or different initial portions of dry mass for different species theoretically. Thus, Eqs. 4 and 6 would be modified to

$$\overline{Y}_{AB\ldots Gt} = p_A \widehat{Y}_{At} + p_B \widehat{Y}_{Bt} + \ldots + p_G \widehat{Y}_{Gt}, \tag{9}$$

and

$$\widehat{Y}_{AB\ldots Gt} = p_A e^{-f_A k_A t} + p_B e^{-f_B k_B t} + \ldots + p_G e^{-f_G k_G t}, \tag{10}$$

where A, B, ..., and G are species in mixed species litters; p_A, p_B, ..., p_G are the proportions of species A, B, ..., and G in the mixtures; and $p_A + p_B + \ldots + p_G = 1$. The idea and method proposed here may work if different decomposition models are used. In the case of a linear model, for example, the exponential model needs to be replaced by the linear model in Eqs. 1, 2, 3, 5, 6. Slopes of the linear model for the observed mixtures and expected mixtures can be compared to detect additive effects. Similarly, enhancement factors can be estimated by fitting the mixed litter with a two parameter linear regression model.

General procedure of data analysis

The analysis includes three steps. (1) Collect litter decomposition data for single species A, B and mixed species AB for a series of time periods. Estimate litter decomposition rates for species A, B and mixture AB (k_A, k_B, and k_{AB}) using Eqs. 1, 2 and 3. Compute 95% confidence intervals of k_A, k_B, and k_{AB}. (2) Calculate expected mass remaining in mixed species litter AB using Eq. 4. Estimate expected litter decomposition rate in the mixture $\left(\overline{k}_{AB}\right)$ using Eq. 5. Detect additive or nonadditive response in mixed species litter by comparing $\overline{k}_{AB}$ and k_{AB}. If $\overline{k}_{AB}$ falls in the 95% confidence interval of k_{AB}, we accept an additive response. If the standard error is large, it may be difficult to find a significant difference between them. (3) Estimate enhancement factors for species A and B using Eq. 6 and test for significant enhancement factors (f ? 1) using Eqs. 7 and 8.

Data analysis can be performed using SAS software (Hui and Jiang 1996; SAS Institute Inc. Cary, NC, USA). PROC NLIN can be used to fit the exponential decay function. Like other nonlinear regression programs, this procedure requires initial values for parameters to be estimated. Initial values of the decomposition rate can be set based on our knowledge of species. For Eq. 6, the initial values for f_A and f_B can be set as 1. If certain prior knowledge of the species response is acquired, e.g., litter decomposition of species A is known to be enhanced in the mixture, this information can be used to constrain the model by setting bounds, e.g., $f_A>1$.

Results

We first test this method using a simulated mass remaining data set, then apply it to a real measurement data set. The simulated data set was created by assuming the litter decomposition follows the exponential decay function with small random error, $Y = \widehat{Y} + e$ (Berges et al. 1994; Böttcher 2004). Mass remaining $\widehat{Y}$ is calculated using Eqs. 1, 2 and 6 given litter decomposition rates for individual species alone and their enhancement factors in the mixture. Random error e is assumed to follow a normal distribution with mean equal to 0, and standard deviation is 5% of the mass remaining $\widehat{Y}$.

Three steps are involved in the validation. (1) We set up different scenarios with given litter decomposition rates and enhancement factors for single species in the mixture (Table 1); (2) We simulate mass remaining for these scenarios using the above method (Table 2); and (3) Based on these simulated data, we estimate litter decomposition rates and enhancement factors, and compare them with initial settings (Tables 3 and 4).

Suppose we have two species A and B, and the litter decomposition rates are 1.5 and 0.8 year^{-1}, respectively (Table 1). Six scenarios (Mixed C, D, E, F, G and H) in the mixtures are simulated with different combinations of enhancement factors for species A and B (Table 1). For example, in scenario Mixed F, litter decomposition of species A is

Table 1 Litter decomposition rates and enhancement factors used in simulating mass remaining data

Parameter	Species A	Species B	Mixed, C	Mixed, D	Mixed, E	Mixed, F	Mixed, G	Mixed, H
k_A	1.5	–	×1.0	×1.6	×0.6	×1.6	×0.6	×1.0
k_B	–	0.8	×1.0	×1.6	×0.6	×0.6	×1.6	×0.6

Values for Species A and B are litter decomposition rates. For mixed litter scenarios, values are enhancement factors for each species.

enhanced by 60% and that of species B is reduced by 40%. We simulate the incubation of litter of species A, B and AB over 2 years, with the mass remaining measured every 2 months (Table 2). Columns Species A and B are fractions of mass remaining for species A and B incubated alone. Mixed C, D, E, F, G and H are six different scenarios of simulated mass remaining in evenly mixed species litter AB.

Detecting additive or non-additive responses in mixed species litter

Based on Eqs. 1 and 2, the decomposition rates in species A and B are calculated as 1.490 and 0.772 year^{-1}, respectively (Table 3), which are not significantly different from the parameters in Table 1. To calculate the expected mass remaining in the mixtures, we apply Eq. 4 to Species A and B (Table 2). Since scenario Mixed C is defined as no change in litter decomposition rate for species A and B in the mixtures, not surprisingly, the calculated expected mass remaining from Species A and B is the same as in Mixed C (Table 2). Applying Eq. 3 to Mixed C (Table 2), we estimate that the expected decomposition rate in mixed species litter as 1.058 year^{-1} (Table 3). Similarly, the decomposition rates in scenarios Mixed D, E, F, G and H are estimated using Eq. 3. Comparing these decomposition rates to the expected decomposition rate from Mixed C (1.058 year^{-1}) by checking if the expected decomposition rate fall in the 95% confidence intervals, we find additive responses in scenarios Mixed C, F and G, a synergistic response in scenario Mixed D, and antagonistic responses in scenarios Mixed E and H.

The commonly used method by averaging two decomposition rates could produce very different conclusions. The expected decomposition rate in the mixtures would be 1.131 year^{-1} averaged by species A and B. While the conclusions for scenarios Mixed D, E and G do not change in our analysis, the additive responses in scenarios Mixed C and F showed here would be detected as non-additive, antagonistic responses. It is apparent that using the mean of decomposition rate overestimates the expected litter decomposition rate and may detect an additive response (e.g., scenarios Mixed C and F here) as an antagonistic response.

Table 2 Simulated fractions of mass remaining in single species and mixed species litter bags with six different scenarios

Time (year)	Species A	Species B	Mixed, C	Mixed, D	Mixed, E	Mixed, F	Mixed, G	Mixed, H
0.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.17	0.768	0.930	0.849	0.761	0.875	0.873	0.797	0.794
0.33	0.627	0.797	0.712	0.519	0.791	0.620	0.611	0.717
0.50	0.445	0.719	0.582	0.424	0.723	0.530	0.618	0.6348
0.67	0.396	0.608	0.502	0.334	0.650	0.473	0.476	0.566
0.83	0.283	0.492	0.387	0.229	0.562	0.407	0.394	0.480
1.00	0.225	0.448	0.337	0.182	0.508	0.361	0.358	0.420
1.17	0.170	0.436	0.303	0.142	0.451	0.317	0.277	0.320
1.33	0.138	0.349	0.243	0.116	0.415	0.296	0.232	0.344
1.50	0.112	0.299	0.205	0.085	0.374	0.241	0.181	0.284
1.67	0.085	0.248	0.167	0.071	0.303	0.232	0.187	0.232
1.83	0.066	0.248	0.157	0.053	0.302	0.226	0.142	0.222
2.00	0.051	0.206	0.129	0.045	0.276	0.186	0.115	0.170

The values are simulated based on the parameters set in Table 1.

Table 3 Parameter estimation of litter decomposition rate in single species and mixed species litter bags

Parameter	Species A	Species B	Mixed, C	Mixed, D	Mixed, E	Mixed, F	Mixed, G	Mixed, H
k	1.490	0.772	1.058	1.712	0.671	1.003	1.099	0.880
s_k	0.020	0.018	0.011	0.031	0.008	0.045	0.032	0.022
Lower 95% CI	1.446	0.733	1.034	1.645	0.654	0.905	1.030	0.832
Upper 95%CI	1.535	0.812	1.083	1.778	0.687	1.102	1.168	0.929
Expected k	1.131[a]				1.058			
Overall response			Additive[b]	Synergistic	Antagonistic	Additive[b]	Additive	Antagonistic

The overall response is determined by comparing if the expected litter decomposition rate (k) falls in the 95% CI of observed k in the mixtures.

[a] Calculated using rate averaging method

[b] If the expected decomposition rate in the mixtures of 1.131 is used, it is antagonistic response for Scenarios C and F.

Partitioning single species contributions in mixed litters

Using Eq. 6, we estimate enhancement factors for species A and B in all scenarios Mixed C to H (Table 4). These estimations are not significantly different from parameters in Table 1. As expected, we do not find a significant difference in the enhancement factor from 1 in scenario Mixed C for either species A or B, confirming the additive response detected above. In scenario Mixed D, the overall synergistic response detected above is caused by a 61.4% significant enhancement in litter decomposition rate of species A and a 60.4% enhancement of species B. In scenario Mixed E, the overall antagonistic response is caused by significantly reduced litter decomposition rates in species A and B in mixed litter. In scenario Mixed F, litter decomposition of species A is significantly enhanced (53.8%), but decomposition of species B is significantly reduced (38.3%); thus the overall response in mixed litter is additive. In scenario Mixed G, the additive response is caused by a significantly reduced decomposition in species A and a significantly enhanced decomposition in species B. In scenario Mixed H, the antagonistic response is caused only by a significantly reduced decomposition in species B (29.4%).

Application

We apply the above methods to litter decomposition data of *Bartsia alpine* and *Betula nana* (Quested et al. 2005). Mass loss from field-incubated litterbags containing *Bartsia* and *Betula* and mixtures of these two species were shown in Fig. 3a and b of Quested et al. (2005). The fraction of mass loss was measured

Table 4 Estimation of enhancement factor in decomposition rate for species A and B in mixed species litters

Species	Enhancement factor	Mixed, C	Mixed, D	Mixed, E	Mixed, F	Mixed, G	Mixed, H
A	f_A	0.843	1.614	0.574	1.538	0.515	0.930
	s_{fA}	0.112	0.129	0.082	0.142	0.071	0.128
	Lower 95%CI	0.597	1.330	0.393	1.225	0.360	0.648
	Upper 95%CI	1.089	1.898	0.754	1.852	0.671	1.211
	t_A	−1.42	4.75[a]	−5.18[a]	3.78[a]	−6.86[a]	−0.55
	Response	No change	Enhanced	Reduced	Enhanced	Reduced	No change
B	f_B	1.130	1.604	0.658	0.617	2.050	0.716
	s_{fB}	0.141	0.098	0.101	0.046	0.328	0.094
	Lower 95%CI	0.820	1.387	0.437	0.517	1.327	0.510
	Upper 95%CI	1.441	1.821	0.880	0.718	2.773	0.922
	t_B	0.92	6.13[a]	−3.39[a]	−8.39[a]	3.20[a]	−3.02[a]
	Response	No change	Enhanced	Reduced	Reduced	Enhanced	Reduced

Student t test is used to test if enhancement factor is significant different with 1.

[a] Indicates significant at α=0.05 level

seven times in 102 weeks. We digitalized litter mass loss in Fig. 3 using Sigma Scan Pro (Systat Software Inc.) and converted to mass remaining. Using Eqs. 1 and 2, we estimate the litter decomposition rates for *Bartsia* and *Betula* to be 0.693±0.078 and 0.207±0.018 year^{-1}, respectively. Litter decomposition rate for the mixture is estimated as 0.423±0.041 year^{-1}, which is not significantly different from the expected litter decomposition rate 0.406 year^{-1} estimated using Eq. 5. Thus, an additive effect of these two species is detected, which is consistent with the conclusions of Quested et al. (2005). We further estimate the enhancement factors for *Bartsia* and *Betula* as 1.601±0.524 (t=1.15, p>0.05) and 0.314±0.481 year^{-1} (t=−1.43, p>0.05). Both of them are not significantly different from 1, indicating that litter decomposition rates of *Bartsia* and *Betula* are not changed in the mixture. It should be noted that the error estimates of enhancement factors are often very high, and further study is needed to confirm these results.

Discussion

We propose a framework to assess the interactive responses of different species in litter mixtures and to partition the contributions of single species in mixed species litter. Using a simulated mass remaining data set with six different scenarios for mixed species litter, we demonstrate that not only can the overall additive or non-additive responses in mixed species litters be detected, but we can also partition the single species contribution to the overall responses. We find that additive responses in the mixtures can be caused by no changes in the decomposition rates of all species (no interaction), or enhanced decomposition in one species and reduced decomposition in another (canceled positive and negative interaction). Similar antagonistic responses in mixed species litter can also be caused by two different mechanisms: a reduced decomposition rate of one species only (Scenario Mixed H) or reduced decomposition of both species (Scenario Mixed G).

The expected litter decomposition rate in mixed species litter calculated here can be quite different from the mean of single species decomposition rates. The difference of the expected litter decomposition rate between our method and the rate averaging method (McArthur et al. 1994; Swan and Palmer 2004; Schweitzer et al. 2005; LeRoy and Marks 2006; Dearden et al. 2006; Taylor et al. 2007; Kominoski et al. 2007) is that their method would calculate the expected mass remaining in the mixtures at time t as

$$\dddot{Y}_{\mathrm{AB}t} = e^{-\frac{k_{\mathrm{A}}+k_{\mathrm{B}}}{2}t} = \sqrt{e^{-k_{\mathrm{A}}t}e^{-k_{\mathrm{B}}t}} \tag{11}$$

compared with our estimate

$$\overline{Y}_{\mathrm{AB}t} = \left(e^{-k_{\mathrm{A}}t} + e^{-k_{\mathrm{B}}t}\right)/2. \tag{12}$$

This latter value represents the expected mass remaining of mixed species litters when there is no interaction between the two single species.

Further partitioning of contributions of single species in the mixtures is quite helpful in distinguishing different mechanisms underlying the overall responses. Since different combinations of single species decomposition rate changes could produce similar overall responses, it is quite important to differentiate the single species contributions in the mixtures. As demonstrated here, for example, an overall additive response in mixed species litter could be the result of no changes in decomposition rate for all species, or an enhanced decomposition of one species and a reduced decomposition of another. Indeed, in mixtures of chestnut oak, red maple and yellow poplar litters, overall mass loss did not differ from expected values (Mudrick et al. 1994), though when oak decaying in mixture was compared to oak decaying alone, it was observed that oak leaves lost more mass when in the mixtures.

Assessing interactive responses in litter decomposition in mixed species litter is an important, yet very difficult task. Statistical analysis provides an objective way of assessing the overall responses and most possible outcomes of single species contributions in the mixtures. Like other regression analyses, random experimental error could significantly influence the parameter estimations (Berges et al. 1994; Böttcher 2004). If we increase random error when simulating the mass remaining data, the standard error of estimated litter decomposition rate would increase. For example, when we assume the standard deviation of random error is 15% of mass remaining, the litter decomposition rates are estimated as 1.470±0.060 and 0.7217±0.051 year^{-1} for species A and B, respectively. Standard errors of decomposition rate estimation are larger for both species A and B. Standard errors of enhancement factors also increase remarkably, making it difficult to detect some significant changes in enhancement factors. For example, enhancement factors for species A and B in scenario Mixed F are estimated as $f_A = 1.404 \pm 0.407$ (t=0.99, p>0.05) and $f_B = 0.646 \pm 0.154$ (t= 2.30, p<0.05), where f_A is not significantly different from 1 (f_A is set as 1.6 in scenario Mixed F). Thus, efforts should be made to lower the random error by improving measurement accuracy, e.g., measuring more litter bags at each sampling date.

Another issue is the number of sampling dates. We test here the duration of the experiment and the frequency of sampling on litter decomposition estimation by using partial simulated mass remaining data. If only the first year of mass remaining is used in parameter estimation, the estimated litter decomposition rates for species A and B are 1.491±0.035 and 0.759±0.035 year^{-1}, respectively. When mass remaining measured at every 4 months is used, we estimate that litter decomposition rates are 1.539± 0.021 and 0.762±0.027 year^{-1} for species A and B, respectively. It seems that long term and frequent sampling produces smaller standard error estimation. Similar studies have also shown that duration of litter incubation could influence the decomposition rate estimation (Böttcher 2004; Prescott 2005). Johnson (1992) also showed that increasing the number of independent data points gives better results than increasing the number of replicates at a single measurement point for non-linear fitting procedures.

Many factors influence the overall response and the contributions of single species in the mixtures. Physical, chemical, and biological processes, individually or in combination, contribute to the overall decomposition process (Swift et al. 1979; Gartner and Cardon 2004). To detect interaction responses in mixed species litter, most of the methods need to estimate the expected litter remaining in the mixtures. The common method is to calculate the mean of mass remaining in single species litter decomposing alone (Wardle et al. 2003; Gartner and Cardon 2004). Ostrofsky (2007) recently proposed to use a double exponential function to estimate expected litter remaining in the mixtures assuming each of the two species follows an exponential decay function. The difference between these two methods is that Ostrofsky's method does not consider the residual errors of single species' model fitting (Eq. 12) which can be estimated as $e_{At} = Y_{At} - e^{-k_A t}$ and $e_{Bt} = Y_{Bt} - e^{-k_B t}$ for species A and B, respectively. A similar method to Ostrofsky's was applied by Schädler and Brandl (2005) who used decomposition rate of individual species without other species to estimate expected litter remaining in the mixtures, but the decomposition rates for single species were estimated using only two points or so.

Analysis of variance (ANOVA) is still widely used in data analysis. But it has been pointed out that the responses of litter decomposition in mixed species litter may have significant interactive effects with the time duration of the experiments (Gartner and Cardon 2004; Ostrofsky 2007). When this interactive effect is significant, the ANOVA method may not provide an accurate description of the litter decomposition response. The method proposed here considers all measured mass remaining data simultaneously and provides a better indicator of litter decomposition response over the ANOVA method. But estimating the decomposition rate requires a series of accurate mass remaining measurements through time, and many studies only conduct two or three measurements of mass remaining (i.e., harvest times) over the study period, preventing the use of this method (Wardle et al. 2003; Vasconselos and Laurance 2005). Whenever possible, long-term measurements of mass remaining at more sampling times are needed in future litter decomposition studies.

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