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# Non-additive effects of water availability and litter quality on decomposition of litter mixtures

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Non-additive (synergistic or antagonistic) effect, a common phenomenon for the decomposition of mixed litter in nature, is usually regulated by litter quality and environmental factors. In this study, we investigated decomposition rates and nutrient (C, N, and P) dynamics in response to water availability in six litter treatments using plant material from Dongting Lake, China. Three single-litter treatments (leaves of *Carex brevicuspis*, leaves of *Miscanthus sacchariflorus*, and stems of *M. sacchariflorus*) and three mixed-litter treatments (1:1 mixtures of single litters) were incubated at three levels of water availability (20%, 46%, and 100% saturation) for 120 days in a mesocosm experiment. Decomposition rates for single-litter treatments were ranked: *M. sacchariflorus* leaves > *C. brevicuspis* leaves > *M. sacchariflorus* stems. Decomposition rates generally increased with increasing water availability. Antagonistic or additive interactions occurred in the *M. sacchariflorus* leaves + *M. sacchariflorus* stems treatment, and synergistic interactions occurred in the other two mixed-litter treatments. N content and lignin loss rate of *M. sacchariflorus* leaves and *M. sacchariflorus* stems were increased by mixing with *C. brevicuspis* leaves. The magnitude of synergistic interactions increased with increasing water availability and the opposite was true for antagonistic interactions. These data suggest that the direction of non-additive effects is dependent on litter quality, while the magnitude is regulated by water availability.

**Keywords:** litter decomposition; synergism; antagonism; water availability; *Carex brevicuspis*; *Miscanthus sacchariflorus*

## Introduction

Litter decomposition plays a critical role in regulating the buildup of soil organic matter in ecosystems and it releases nutrients for plant growth and influences carbon cycling (Jiang et al. 2013). Studies using mixed litter from multiple species, genotypes, phenotypes, or plant parts have shown that decomposition of litter mixtures at times cannot be predicted from single litters because of non-additive effects (synergistic or antagonistic interactions; Schweitzer et al. 2005). Non-additive interactions are usually caused by the stimulatory or inhibitory effects of nutrient and/or secondary compound (e.g., tannins, simple phenolics) transfer among litter types (Taylor et al. 2007; Tiunov 2009). Synergistic interactions depend on the nutrient status of labile organic matter or on differences in the initial nutrient content of litter types (Liu et al. 2007; Schimel & Hättenschwiler 2007). Antagonistic interactions often occur in litter with low nutrient content (e.g., N, P,

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soluble substances, non-lignified carbohydrates) and high content of secondary compounds (Jiang et al. 2013). The overall effect of mixtures on decomposition depends on the balance between these two interactions (Li et al. 2013).

The magnitude of non-additive effects is usually defined as the difference between observed and expected values (Duan et al. 2013). The direction and magnitude of non-additive effects depend on litter properties, incubation time, soil biota, and microenvironment (e.g., temperature, exogenous nutrients; Gonçalves & Canhoto 2009; Duan et al. 2013; Jiang et al. 2013). In wetlands, water availability regulates the activity of decomposer communities and leaching processes (Clein & Schimel 1994) and litter decomposition rates can increase with increasing water availability (Liu et al. 2006). Studies using litter mixtures suggest that water can affect interactions among litter components by leaching and passive diffusion (Briones & Ineson 1996). In addition, active nutrient exchange occurs via fungal hyphae, the activity of which is limited by water availability (Xu et al. 2010). Although water can play an important role in the exchange of nutrients and secondary compounds, it is unclear whether water availability affects the interactions between components of mixed litter.

In this study, non-additive effects in response to water availability were investigated in litter types of different quality from Dongting Lake, the second largest lake in China. Three types of single litter (leaves of *Carex brevicuspis*, leaves of *Miscanthus sacchariflorus*, and stems of *M. sacchariflorus*) and three types of litter mixtures (combinations of two single litter components) were incubated at three levels of water availability. *C. brevicuspis* and *M. sacchariflorus* are dominant macrophyte species in the Dongting Lake wetlands, where they occur in different elevation and soil moisture zones. *C brevicuspis* prefers lower elevations (higher soil moisture), while *M. sacchariflorus* is found in habitats at higher elevation and with lower soil moisture content (Chen et al. 2014; Pan et al. 2014). These species also coexist in some areas and litter mixtures occur in these areas. Our preliminary analyses showed that initial N and P contents were highest in *C. brevicuspis* leaves and lowest in *M. sacchariflorus* stems. Here, we tested the following hypotheses: (1) decomposition rates of both single and mixed litter will increase with increasing water availability; (2) synergistic interactions will occur in litter mixtures that include *C. brevicuspis* leaves because of the high nutrient content and antagonistic interactions will occur in mixtures containing *M. sacchariflorus* leaves and stems because of the low nutrient and high lignin content; and (3) the magnitude of non-additive effects will increase with increasing water availability because of transfer of nutrients and secondary compounds.

## Methods

### *Collection and preparation of litter*

We collected *C. brevicuspis* leaf litter and *M. sacchariflorus* leaf and stem litter from standing dead plants at Dongting Lake (29° 27′ 2.02″ N, 112° 47′ 32.28″ E), Hunan, China, in November 2012. After collection, the leaves and stems were air-dried to constant mass for 48 hr and cut into pieces (∼10 cm). Weighed litter samples (5 g), including three single and three mixed (1:1 mixtures, by weight, of single components) litters, were placed into 10 × 15-cm nylon bags (1-mm mesh). This mesh size would exclude macroinvertebrates but allow microbial colonization and leaching of litter fragments (Langhans et al. 2008). Sets (strings) of six bags (one of each litter type) were strung together by wire cables to facilitate harvest.

### *Experimental set-up*

The experimental treatments consisted of three levels of water availability in a one-way factorial design. Strings of litterbags ($n = 45$, three per harvest) were placed in mesocosm tanks ($1 \times 3 \times 0.7$ m) in March 2013 at the Dongting Lake Station for Wetland Ecosystem Research. Three mesocosms were divided evenly into three sections, each of which was then filled with 0.2, 0.4, or 0.6 m of sand and water depth was maintained at 0.2 m; moisture content at 5 cm depth (relative to sand surface) was 100% (high water availability), 46% (medium water availability), and 10% (low water availability), respectively. Washed sand was chosen as a substrate to avoid confounding effects of environmental nutrients on litter decomposition (Sylvain et al. 2011). Moisture content was maintained at initial levels by adding water every 2 d.

Litter strings were randomly placed in the mesocosms (at 5-cm intervals) on 14 April 2013 and the litterbags were buried at 5 cm depth in the sand. A total of 270 litterbags were used (3 replicates $\times$ 6 litter types $\times$ 3 treatments $\times$ 5 harvests). Three strings per treatment were sampled after 15, 30, 60, 120, and 240 d of incubation. Prior to incubation, three samples of each single litter were prepared to measure initial litter quality.

### *Chemical analyses*

After collection, litter samples were separated and washed gently using deionized water until the water was transparent and then oven-dried at 60 °C to constant weight (1 wk) to measure remaining dry mass (accuracy to 0.01 g). All samples were ground to powder and passed through a 0.5-mm mesh screen for analysis of litter quality. Samples of initial litter were analyzed for N, P, organic C, cellulose, and lignin contents; samples of incubated litter were analyzed for N, P, and lignin contents. Organic C content was analyzed using the $H_2SO_4{-}K_2Cr_2O_7$ heat method, N and P were quantified using Kjeldahl digestion followed by colorimetric analysis, and cellulose and lignin contents were measured by hydrolysis (10% $H_2SO_4$ for cellulose, 72% $H_2SO_4$ for lignin; Graça et al. 2005).

### *Calculation and statistical analyses*

Decomposition rate ($k$) for each litter type was calculated as follows:

$$-kt = \ln(W_t/W_0),$$

where $W_0$ is the initial litter mass and $W_t$ is the mass remaining at time $t$ in days (Olson 1963; Gingerich et al. 2014). Mass remaining was calculated as a percentage of the initial mass. Components (N, P, and lignin) that remained in litter were determined by multiplying litter mass by the content of each component. Decomposition rate, mass remaining, and litter components were compared among the single-litter treatments by two-way analysis of variance (ANOVA) with treatment and time as main factors.

The expected mass that remained in mixed-litter treatments was estimated based on the mass remaining in single-litter bags from the same string as follows (Hoorens et al. 2003):

$$\text{Expected mass remaining} = (R_1 + R_2)/2,$$

where $R_1$ and $R_2$ indicate the mass remaining in litterbags containing single litters. Litter interactions were defined as deviations between the observed and expected mass remaining. Zero indicated an additive interaction; positive and negative values indicated synergistic and antagonistic interactions, respectively.

Paired *t*-tests were used for the three litter treatments to assess whether observed and expected mass remaining and components differed. Then, analysis of covariance (ANCOVA) was used to test whether the magnitude of litter interaction depended on water availability, with expected mass remaining as a covariate (Duan et al. 2013). All statistical analyses were performed using SPSS version 21.

## Results

### Initial litter quality

Initial N, P, organic C, cellulose, and lignin contents differed among the three litter treatments ($p < 0.05$; Table 1). N and P contents were highest in *C. brevicuspis* leaves, intermediate in *M. sacchariflorus* leaves, and lowest in *M. sacchariflorus* stems ($p < 0.05$); lignin content showed the opposite trend ($p < 0.05$). The initial ratios of C:N, C:P, and lignin:N were lowest in *C. brevicuspis* leaves and highest in *M. sacchariflorus* stems ($p < 0.05$; Table 1). Differences in nutrient contents among the treatments were largest in the *C. brevicuspis* leaves + *M. sacchariflorus* stems mixture, intermediate in *C. brevicuspis* leaves + *M. sacchariflorus* leaves, and lowest in *M. sacchariflorus* leaves + *M. sacchariflorus* stems ($p < 0.05$).

### Decomposition of single-litter treatments

All single-litter types decayed most quickly in the initial two weeks of the experiment, after which decomposition rates slowed down (Figure 1). Decomposition rates of single litters were highest in *M. sacchariflorus* leaves at high water availability and lowest in *M. sacchariflorus* stems at low water availability (Table 2). Two-way ANOVA showed significant effects of water availability on decomposition rate for all single-litter treatments ($p < 0.001$; Table 3).

Table 1. Initial quality of the three types of plant litter used in the study.

| | Litter type | | |
|---|---|---|---|
| Parameter | *Carex brevicuspis* leaves | *Miscanthus sacchariflorus* leaves | *Miscanthus sacchariflorus* stems |
| N (mg $g^{-1}$) | 7.68 ± 0.18a | 4.15 ± 0.85b | 1.40 ± 0.25c |
| P (mg $g^{-1}$) | 0.89 ± 0.10a | 0.48 ± 0.13b | 0.14 ± 0.02c |
| Organic C (%) | 38.37 ± 1.77c | 43.13 ± 0.85b | 49.12 ± 1.70a |
| Cellulose (%) | 14.61 ± 0.31a | 18.56 ± 2.53b | 18.48 ± 0.14c |
| Lignin (%) | 30.75 ± 1.41a | 32.42 ± 0.91b | 34.47 ± 2.64c |
| C:N (g $g^{-1}$) | 50.02 ± 3.26a | 107.73 ± 27.30b | 359.86 ± 83.73c |
| C:P (g $g^{-1}$) | 433.9 ± 27.85a | 943.5 ± 230.7b | 3534. ± 605.0c |
| N:P (g $g^{-1}$) | 8.72 ± 1.09b | 8.87 ± 1.94a | 9.97 ± 1.67c |
| Lignin:N (g $g^{-1}$) | 40.07 ± 2.09a | 80.99 ± 20.76b | 253.04 ± 63.35c |

All values are means of three replicates, expressed on a dry-mass basis. Different lowercase letters (a, b, c) within rows indicate significant difference in initial litter quality among the three litter types (LSD test, $p < 0.05$).

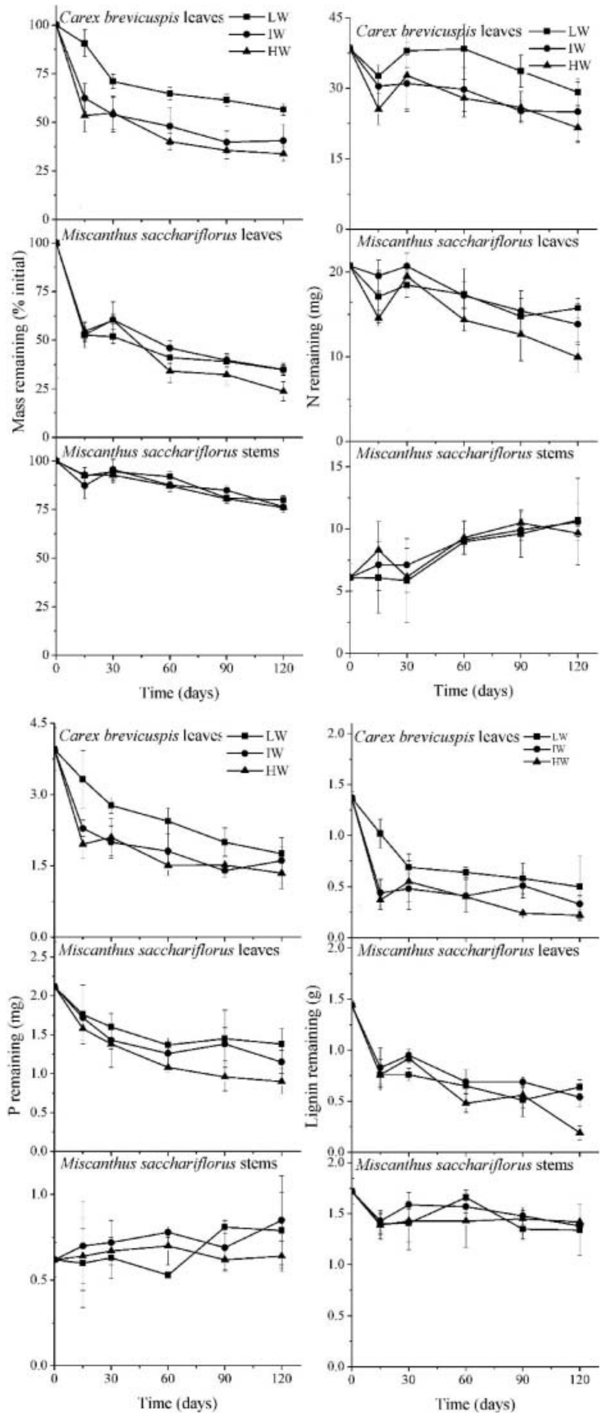


Figure 1. Percentage (mean ± SE) of mass, nitrogen, phosphorus, and lignin remaining in three single litters in three water availability treatments. LW, IW, and HW indicate low, intermediate, and high water availability, respectively.

Table 2. Regression statistics ($r^2$) for exponential rates of decomposition ($k$).

| | *C. brevicuspis* leaves | | *M. sacchariflorus* leaves | | *M. sacchariflorus* stems | |
|---|---|---|---|---|---|---|
| Treatments | $k$ | $r^2$ | $k$ | $r^2$ | $k$ | $r^2$ |
| LW | 0.0046 | 0.83 | 0.0070 | 0.70 | 0.0018 | 0.80 |
| IW | 0.0066 | 0.66 | 0.0072 | 0.76 | 0.0018 | 0.65 |
| HW | 0.0077 | 0.71 | 0.0108 | 0.83 | 0.0022 | 0.94 |

$k$ is a decomposition constant ($d^{-1}$) based on an exponential model. LW, IW, and HW indicate low, intermediate, and high water availability, respectively.

Table 3. One-way ANOVA results of mass, N, P, and lignin remaining in single litters in single- and mixed- litter bags as well as ratio of lignin:N, lignin:P, and N:P in mixed- litter bags in three water availability treatments (df = 2).

| | *F*-value | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Mass | 19.94** | 3.95* | 3.35* | 9.70** | 8.19** | 9.93** |
| N | 10.03** | 4.41* | 1.26 | 20.83** | 2.72 | 3.91* |
| P | 10.49** | 5.51** | 2.63 | 11.77** | 4.43* | 7.19** |
| Lignin | 13.67** | 2.32 | 2.97 | 13.17** | 6.39** | 12.80** |
| Lignin:N | | | | 3.18* | 1.52 | 3.35* |
| Lignin:P | | | | 3.26* | 1.38 | 0.31 |
| N:P | | | | 0.37 | 0.12 | 0.56 |

* $p < 0.05$; ** $p < 0.01$. The letters A, B, C, D, E, and F indicate *Carex brevicuspis* leaves, *Miscanthus sacchariflorus* leaves, and *Miscanthus sacchariflorus* stems, and litter mixtures of *Carex brevicuspis* leaves + *Miscanthus sacchariflorus* leaves, *Carex brevicuspis* leaves + *Miscanthus sacchariflorus* stems, and *Miscanthus sacchariflorus* leaves + *Miscanthus sacchariflorus* stems.

Both leaf litters released N and P at the end of incubation, but *M. sacchariflorus* stems accumulated these nutrients (Figure 1). P content decreased more rapidly than N content in both leaf litters. The release of N and P from leaf litter was significantly promoted by increased water availability ($p < 0.01$) but N and P accumulation in *M. sacchariflorus* stems was not affected by water availability ($p > 0.05$). The effects of water availability on lignin content of the single-litter treatments were significant ($p < 0.05$) and lignin content decreased more quickly in leaf litter than in *M. sacchariflorus* stems.

### *Decomposition of mixed litters*

The effects of water availability on decomposition rates of the three litter mixture treatments were significant ($p < 0.001$; Figure 2). Similar to single-litter types, decomposition of mixed litters was most rapid initially and was enhanced by increased water availability. For a given level of water availability, decomposition rate among the mixed litters was, from fastest to slowest: *C. brevicuspis* leaves + *M. sacchariflorus* leaves > *C. brevicuspis* leaves + *M. sacchariflorus* stems > *M. sacchariflorus* leaves + *M. sacchariflorus* stems.

Similar to single-litter types, the leaf litters in mixtures released N and P, while the stem litter accumulated these nutrients at the end of the incubation (Figures 3 and 4). N content of *M. sacchariflorus* leaves and *M. sacchariflorus* stems was higher when mixed with *C. brevicuspis* leaves ($p < 0.05$; Table 4). P content of *M. sacchariflorus* leaves was lower when mixed with *C. brevicuspis* leaves ($p < 0.05$). Lignin loss rates of

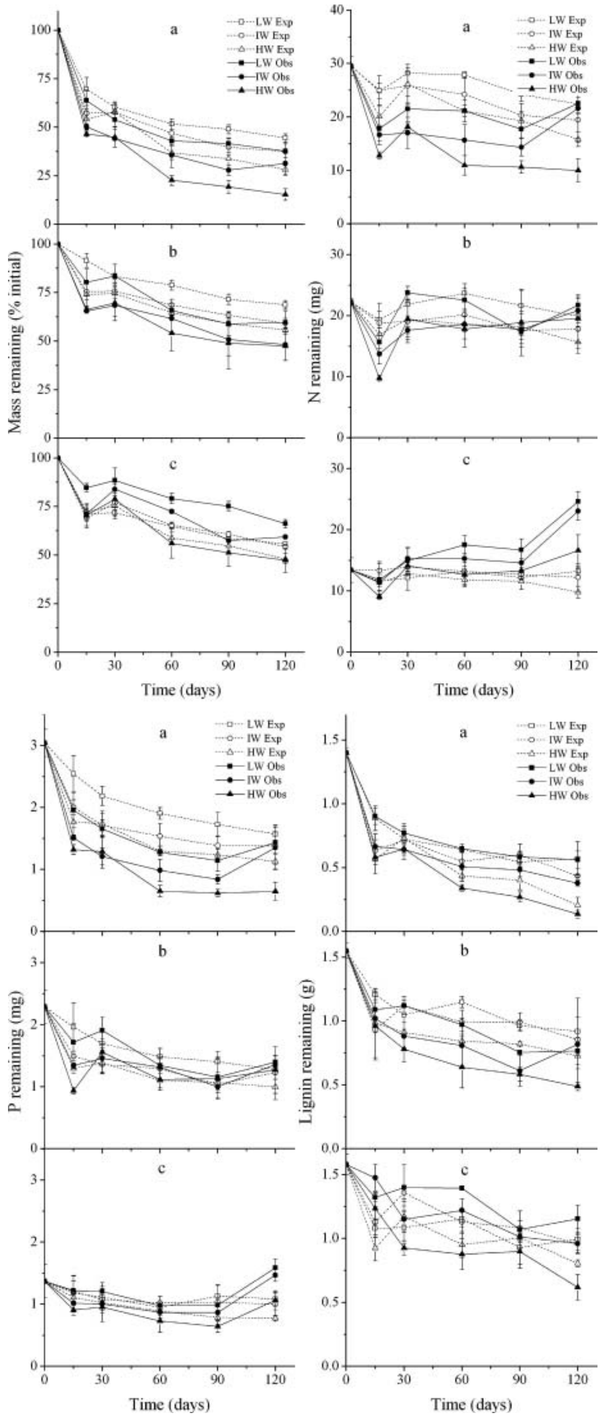


Figure 2. Expected (Exp) and observed (Obs) mass, nutrients, and lignin remaining in the three litter mixtures in the three water availability treatments (mean ± SE). LW, IW, and HW indicate low, intermediate, and high water availability, respectively. The letters a, b, and c indicate *C. brevicuspis* leaves + *M. sacchariflorus* leaves, *C. brevicuspis* leaves + *M. sacchariflorus* stems, and *M. sacchariflorus* leaves + *M. sacchariflorus* stems mixture, respectively.

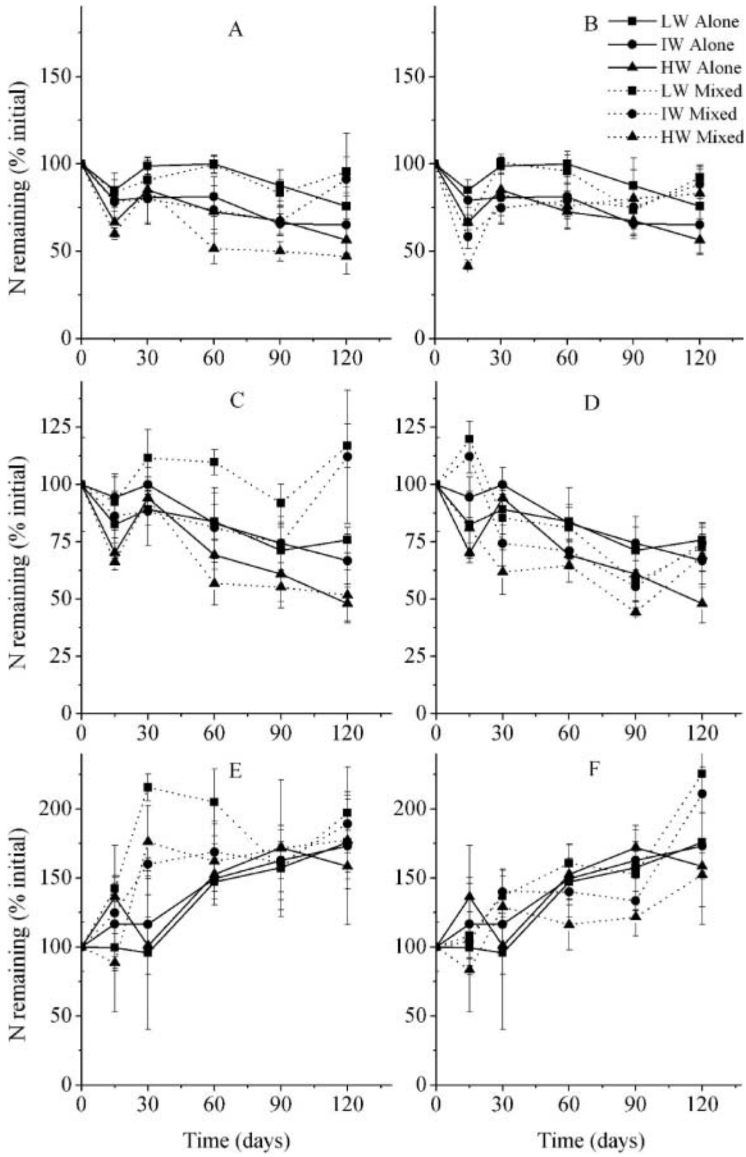


Figure 3. N remaining in litter according to mixture types (mean ± SE). LW, IW, and HW indicate low, intermediate, and high water availability, respectively. The letters A, B, C, D, E, and F indicate *C. brevicuspis* leaves mixed with *M. sacchariflorus* leaves and *M. sacchariflorus* stems, *M. sacchariflorus* leaves mixed with *C. brevicuspis* leaves and *M. sacchariflorus* stems, and *M. sacchariflorus* stems mixed with *C. brevicuspis* leaves and *M. sacchariflorus* leaves, respectively.

*M. sacchariflorus* leaves were higher when mixed with *C. brevicuspis* leaves but lower when mixed with *M. sacchariflorus* stems (Figure 5, $p < 0.05$). Lignin loss rates of *M. sacchariflorus* stems were higher when mixed with *C. brevicuspis* leaves and mixed with *M. sacchariflorus* leaves at high water availability ($p < 0.05$). Other litter component dynamics were not affected.

The lignin:N and lignin:P ratios for mixtures increased for all mixtures and the N:P ratios decreased (Figure 6). The lignin:N and N:P ratios in the *C. brevicuspis*

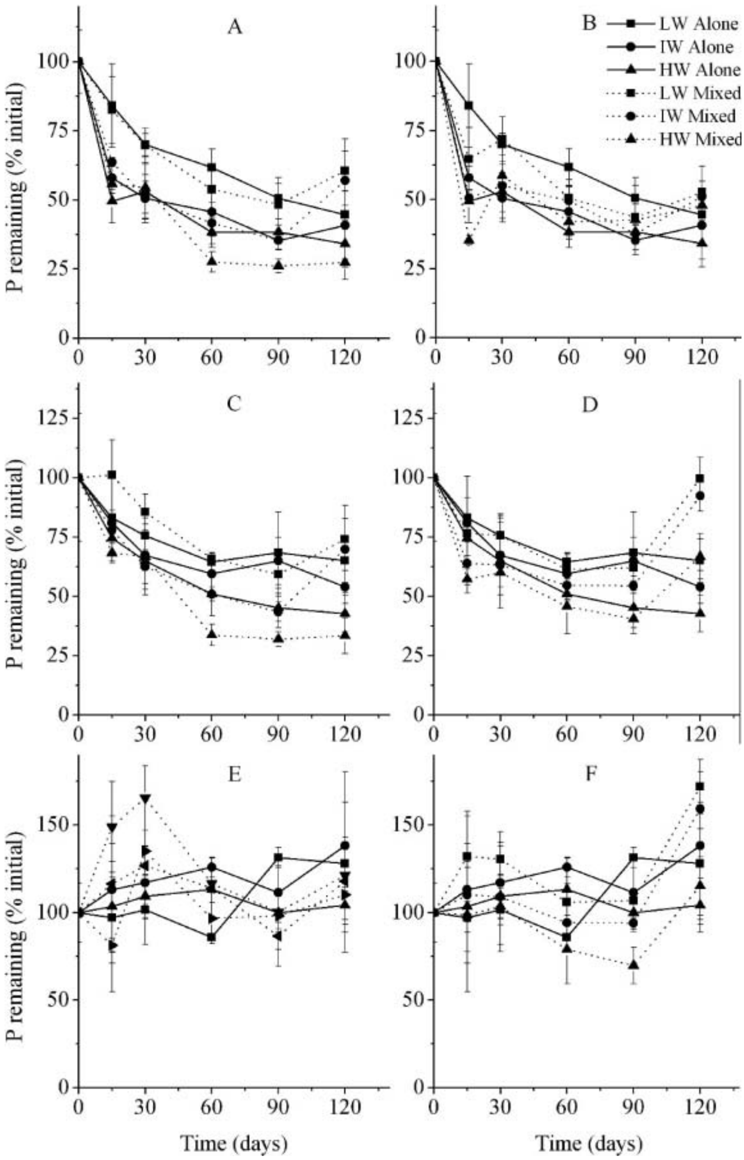


Figure 4. P remaining in litter according to mixture types (mean ± SE). LW, IW, and HW indicate low, intermediate, and high water availability, respectively. The letters A, B, C, D, E, and F indicate *C. brevicuspis* leaves mixed with *M. sacchariflorus* leaves and *M. sacchariflorus* stems, *M. sacchariflorus* leaves mixed with *C. brevicuspis* leaves and *M. sacchariflorus* stems, and *M. sacchariflorus* stems mixed with *C. brevicuspis* leaves and *M. sacchariflorus* leaves, respectively.

leaves + *M. sacchariflorus* treatment and the lignin:N ratio in the *M. sacchariflorus* leaves + *M. sacchariflorus* stems treatment were significantly affected by increasing water availability ($p < 0.01$; Figure 6). Other litter-component ratios were not affected ($p > 0.05$).

Mass remaining of *C. brevicuspis* leaves was not affected by mixing ($p < 0.01$; Table 4 and Figure 7). Mass remaining of *M. sacchariflorus* leaves was lower when mixed with *C. brevicuspis* leaves but higher when mixed with *M. sacchariflorus* stems ($p < 0.01$). Mass

Table 4. Results of *t*-tests between values at decay alone and values at decay mixed of litter mass, nutrients, and lignin remaining for three litter types after incubation (df = 44).

| | Mass | | N | | P | | Lignin | |
|---|---|---|---|---|---|---|---|---|
| Litter type in mixture | *t* | *p* | *t* | *p* | *t* | *p* | *t* | *p* |
| | | | Mixed with *Carex brevicuspis* leaves | | | | | |
| *M. sacchariflorus* leaves | 9.176 | <0.01 | −15.047 | <0.01 | −12.884 | <0.01 | 4.535 | <0.05 |
| *M. sacchariflorus* stems | 11.464 | <0.01 | −3.545 | <0.01 | −0.679 | 0.501 | 33.901 | <0.01 |
| | Mixed with *Miscanthus sacchariflorus* leaves | | | | | | | |
| *C. brevicuspis* leaves | 0.674 | 0.475 | 0.291 | 0.792 | 0.136 | 0.899 | 0.753 | 0.474 |
| *M. sacchariflorus* stems | 0.328 | 0.766 | 1.122 | 0.219 | 0.354 | 0.748 | 0.9530 | 0.336 |
| | Mixed with *Miscanthus sacchariflorus* stems | | | | | | | |
| *C. brevicuspis* leaves | 0.854 | 0.405 | 0.253 | 0.818 | 0.167 | 0.877 | −0.821 | 0.427 |
| *M. sacchariflorus* leaves | −13.883 | <0.01 | −4.602 | <0.05 | −11.385 | <0.01 | −19.338 | <0.01 |

remaining of *M. sacchariflorus* stems was lower when mixed with *C. brevicuspis* leaves and mixed with *M. sacchariflorus* leaves at higher water availability but higher when mixed with *M. sacchariflorus* leaves at low and intermediate water availability ($p < 0.01$). The mixtures of *C. brevicuspis* leaves + *M. sacchariflorus* leaves and *C. brevicuspis* leaves + *M. sacchariflorus* stems decayed faster than expected and the mixture of *M. sacchariflorus* leaves + *M. sacchariflorus* stems decayed more slowly than expected in all but the low-water-availability treatments (Table 5). ANCOVA showed that the magnitude of synergistic interactions increased and that of antagonistic interactions decreased, with increasing water availability (Figure 2 and Table 6). For example, from low to high water availability, litter mass loss was increased from +6.67% to +12.93% for mixtures of *C. brevicuspis* leaves + *M. sacchariflorus* leaves, and was reduced from −10.30% to +0.68% for mixtures of *M. sacchariflorus* leaves + *M. sacchariflorus* stems.

## Discussion

Decomposition rates for all single and mixed litters increased with increasing water availability, which is consistent with our first hypothesis and with the findings of Liu et al. (2006). This facilitation effect of high water availability might result from high decomposer activity in moist substrates because water was essential for microbial growth (Osono et al. 2003). Rapid leaching might be another explanation. Langhans et al. (2008) have observed that high water availability stimulates initial decomposition in *C. brevicuspis* leaves.

Synergistic interactions were found in the mixtures of *C. brevicuspis* leaves + *M. sacchariflorus* leaves and *C. brevicuspis* leaves + *M. sacchariflorus* stems and antagonistic or addictive interactions occurred in the *M. sacchariflorus* leaves + *M. sacchariflorus* stems mixture. These results indicated that the direction of non-additive effects might be determined by litter quality rather than by water availability (Schimel & Hättenschwiler 2007). These results are consistent with our second hypothesis. Synergistic interactions might be attributed to highly efficient translocation of nutrients released from *C. brevicuspis* leaves because a large amount of N and P were released from *C. brevicuspis* leaves in mixture. Antagonistic interactions might be a result of secondary compounds and lower nutrient contents (Hättenschwiler et al. 2005; Liu et al. 2007). Berglund and Ågren

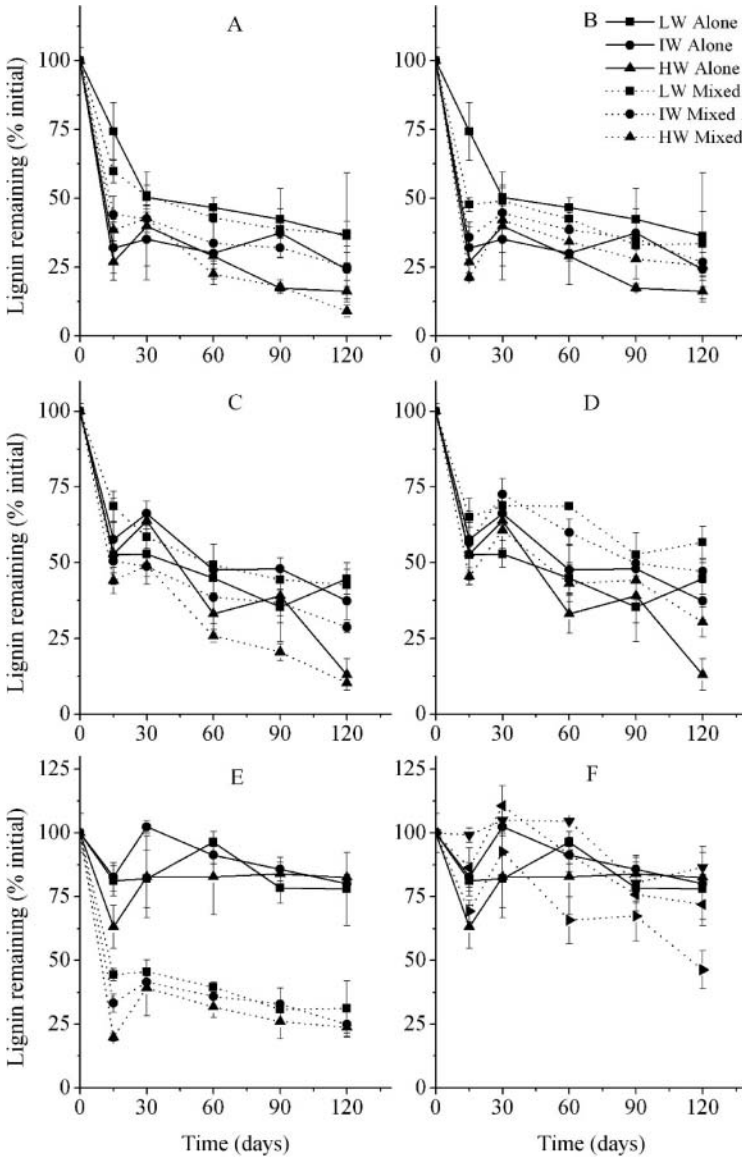


Figure 5. Lignin remaining in litter according to mixture types (mean ± SE). LW, IW, and HW indicate low, intermediate, and high water availability, respectively. The letters A, B, C, D, E, and F indicate *C. brevicuspis* leaves mixed with *M. sacchariflorus* leaves and *M. sacchariflorus* stems, *M. sacchariflorus* leaves mixed with *C. brevicuspis* leaves and *M. sacchariflorus* stems, and *M. sacchariflorus* stems mixed with *C. brevicuspis* leaves and *M. sacchariflorus* leaves, respectively.

(2012) have noted that synergistic interactions occur when high-quality litter releases nitrogen rapidly. After mixed with *C. brevicuspis* leaves, the N and P contents of *M. sacchariflorus* leaves and *M. sacchariflorus* stems were higher than expected with no change in the nutrient dynamics of *C. brevicuspis* leaves, which suggested that a large proportion of released nutrients from *C. brevicuspis* leaves remained in the litter mixture (Liu et al. 2007; Duan 2013). According to the resource-complementarity mechanism for synergistic interactions, nutrient transfer from nutrient-rich litter might be able to satisfy

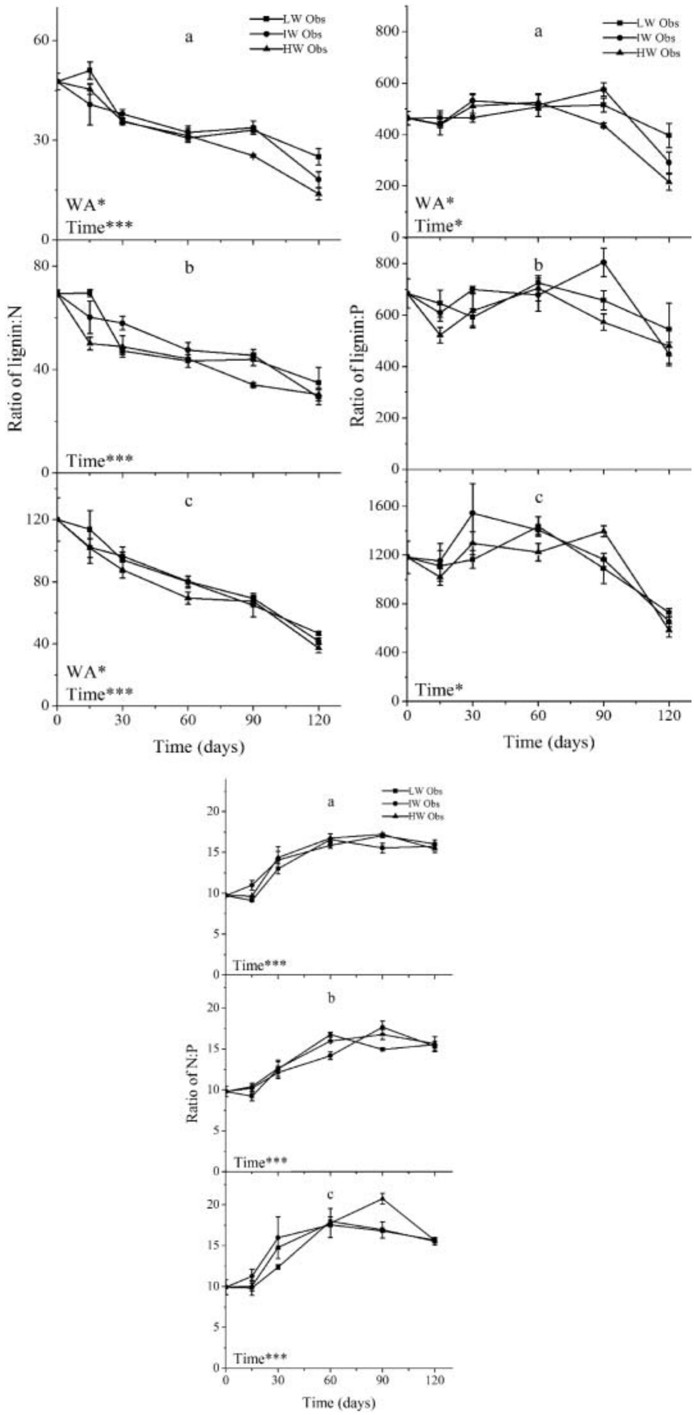


Figure 6. Observed (Obs) lignin:N, lignin:P, and N:P ratios (mean ± SE) in litter mixtures according to water availability. LW, IW, and HW indicate low, intermediate, and high water availability, respectively. The letters a, b, and c indicate mixtures of *C. brevicuspis* leaves + *M. sacchariflorus* leaves, *C. brevicuspis* leaves + *M. sacchariflorus* stems, and *M. sacchariflorus* leaves + *M. sacchariflorus* stems, respectively. $^{*}p < 0.05$; $^{***}p < 0.001$.

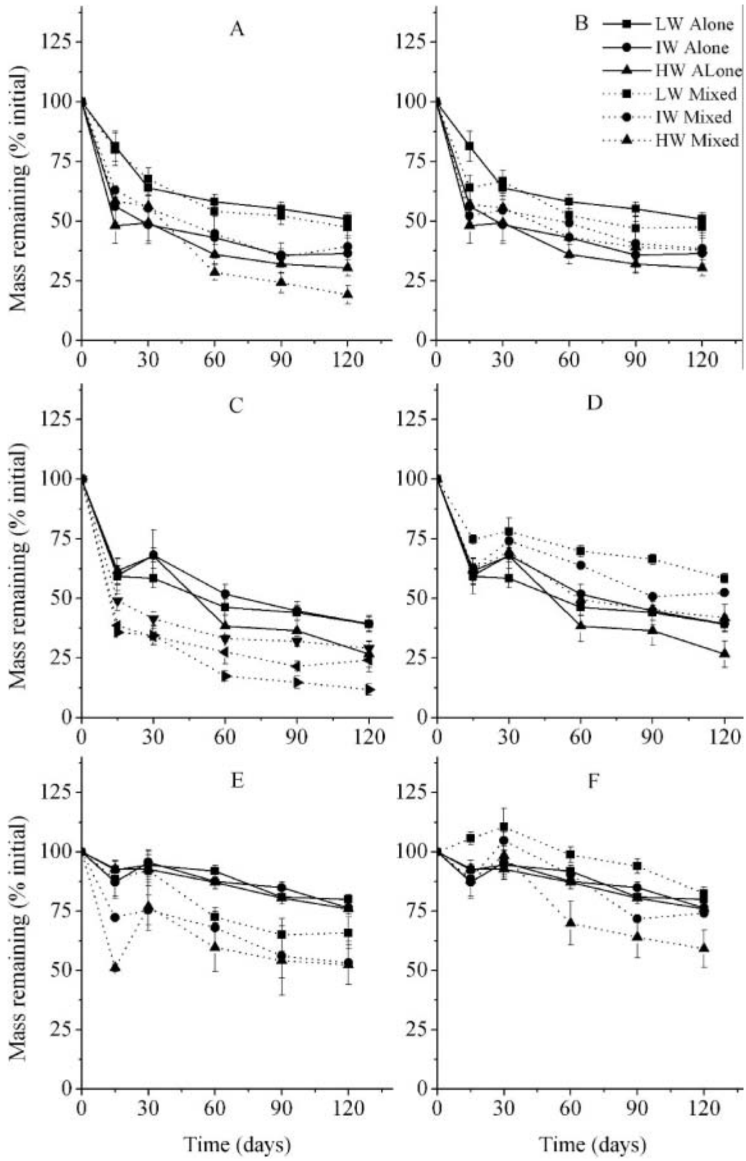


Figure 7. Mass remaining in litter according to mixture types (mean ± SE). LW, IW, and HW indicate low, intermediate, and high water availability, respectively. The letters A, B, C, D, E, and F indicate *C. brevicuspis* leaves mixed with *M. sacchariflorus* leaves and *M. sacchariflorus* stems, *M. sacchariflorus* leaves mixed with *C. brevicuspis* leaves and *M. sacchariflorus* stems, *M. sacchariflorus* stems mixed with *C. brevicuspis* leaves and *M. sacchariflorus* leaves, respectively.

the growth and development requirements of ligninolytic fungi in litters with low nutrient or high organic carbon contents (Gessner et al. 2010). Antagonistic interactions might have been caused by secondary compounds such as phenolics in *M. sacchariflorus* stems (Parveen et al. 2013), which retain N in litter by formation of phenol−protein complexes and inactivation of microbial enzymes (Aerts & Chapin 2000). Accumulation of N and P in stem litter suggested that decomposers could not assimilate adequate nutrients from

Table 5. Results of *t*-tests between expected values and observed values of mass remaining in mixed-litter treatments after incubation (df = 14).

| Mixed litters | LW | | IW | | HW | |
|---|---|---|---|---|---|---|
| | *t*-Value | *p*-Value | *t*-Value | *p*-Value | *t*-Value | *p*-Value |
| *Carex brevicuspis* leaves + *Miscanthus sacchariflorus* leaves | 17.32 | <0.001 | 11.58 | <0.001 | 10.24 | <0.001 |
| *C. brevicuspis* leaves + *M. sacchariflorus* stems | 5.639 | <0.001 | 4.767 | <0.001 | 4.086 | <0.01 |
| *M. sacchariflorus* leaves + *M. sacchariflorus* stems | −12.37 | <0.001 | −3.202 | <0.01 | 0.675 | 0.511 |

LW, IW, and HW indicate low, intermediate, and high water availability, respectively.

Table 6. ANCOVA of magnitude of non-additive effects.

| Mixed litters | Time | | Treatment | |
|---|---|---|---|---|
| | *F*-value | *p*-Value | *F*-value | *p*-Value |
| *Carex brevicuspis* leaves + *Miscanthus sacchariflorus* leaves | 6.496 | <0.001 | 13.100 | <0.001 |
| *C. brevicuspis* leaves + *M. sacchariflorus* stems | 4.426 | <0.01 | 3.696 | <0.05 |
| *M. sacchariflorus* leaves + *M. sacchariflorus* stems | 2.616 | 0.056 | 45.594 | <0.001 |

this litter. This demand would not be satisfied by *M. sacchariflorus* leaves, which released lower quantities of nutrients compared with *C. brevicuspis* leaves. As a result, inhibition by secondary compounds might dominate litter interactions, leading to less lignin loss than expected.

The magnitude of synergistic interactions increased but the magnitude of antagonistic interactions decreased, with increasing water availability, which was partially consistent with our third hypothesis. The promotional effect of high water availability on decomposition might result from leaching of nutrients from *C. brevicuspis* leaves or from stimulation of nutrient transfer, since water is involved in passive diffusion and active transport of nutrients by fungal hyphae (Taylor et al. 2007). Because more nutrients are transported to litter with lower nutrient content under conditions of high water availability, lignin in litter mixtures usually decays faster under these conditions. There are two possible explanations for the weakened antagonistic interactions. First, the facilitative effect of increased water availability is strong enough to override the negative effects of secondary compounds. Similarly, antagonistic interactions of secondary compounds might be eliminated by the activity of soil fauna (Jiang et al. 2013). Second, the improved quality (decreased ratio of lignin:N) of mixed litter at high water availability might offset the inhibitory effects of secondary compounds. The lignin:N ratio was lower at higher water availability, which might result from rapid loss of lignin. This result is consistent with observations by Rosemond et al. (2010) that nutrient enrichment reduced the litter C:N ratio and that the antagonistic interactions disappeared.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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