



Mixing litter from deciduous and evergreen trees enhances decomposition in a subtropical karst forest in southwestern China



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ABSTRACT

In the karst region of southwestern China, mixed evergreen and deciduous broad-leaved forest is typical vegetation, differing remarkably from the evergreen broad-leaved forest developed in non-karst regions in the same latitudinal zone. Litter input from deciduous trees influences the characteristics of the litter layer, which could affect decomposition dynamics. We evaluated the role of deciduous trees in regulating the decomposition of mixed leaf litter in a karst forest. Four species richness treatments (one, two, four and six species) were designed to evaluate the decomposition rates of litter mixtures for two years. The ratio of deciduous to evergreen species numbers was 1:1 in all mixtures. Litter mass loss was positively correlated with initial nitrogen concentrations and negatively correlated with carbon:nitrogen ratios, suggesting that mass loss was controlled by nitrogen concentration of the litter mixture. Litter mixing accelerated the mass loss by 5.0% and enhanced nutrient release by 4.8%–26.2% for different elements. Synergistic effects in the four-species mixture were usually strongest and increased over time. Although individual litter species within the mixtures showed idiosyncratic responses to litter species richness, mixing effects enhanced decomposition of evergreen litter species more than deciduous species. This study suggests that species composition was more important than species richness in driving non-additive effects on decomposition in this forest. Deciduous trees in karst ecosystems significantly contribute to nutrient cycling, through enhancing the decomposition of evergreen leaf litter.

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1. Introduction

Karst topography is widespread globally and covers about 12% of the world's land area (Liu, 2009). The karst landscape of southwestern China, occupying more than 500 000 km², is one of the most typical landscapes developed on carbonate bedrock in the world and is characterized by extremely slow soil formation from the underlying limestone and very shallow and patchy soils with a low water retention capacity (Zhu, 1997; Liu, 2009). It may take more than 10 000 years to form 1 cm of topsoil in the area (Wei, 1996), and the mean depth of topsoil on karst hills is only about

2–9 cm (Zhang and Wang, 2009; Liu et al., 2013). Because plants grow in a limited soil volume, they have access to smaller amounts of nutrients.

The typical vegetation developed on the carbonate bedrock in the subtropical region of China is mixed evergreen and deciduous broad-leaved forest, which remarkably differs from the representative vegetation type in non-karst regions in the same latitudinal zone, the evergreen broad-leaved forest (Guo et al., 2011). Deciduous trees often dominate karst forests due to better adaptive strategies to drought stress than evergreen trees (Liu et al., 2010, 2011a), and play an important role in nutrient cycling of karst ecosystems. For example, leaves from deciduous species accounted for 35%–66% of annual leaf litter fall in karst forests (Wei et al., 2009; Yu et al., 2011). Leaf litter of deciduous species usually shows higher nutrient concentrations (Liu et al., 2014) and specific leaf area than evergreen leaf litter. The large input of litter from

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deciduous trees into this system substantially influences characteristics of the litter layer, which can affect litter decomposition dynamics, such as nutrient transfer among litter types. However, the role of deciduous trees in regulating the decomposition process of mixed litter in karst forests has not been addressed, and is urgently needed to improve our understanding of nutrient cycling, productivity and stability of karst ecosystems.

Litter mixing effects on decomposition may be additive (i.e., decomposition dynamics of a litter mixture can be predicted from single-species dynamics) or non-additive, depending on the ecosystem under investigation (Gartner and Cardon, 2004; Hättenschwiler et al., 2005; Gessner et al., 2010). Several studies have demonstrated that mixing litter can either accelerate or decelerate decomposition (Hector et al., 2000; Gartner and Cardon, 2004; Hättenschwiler and Gasser, 2005; Hättenschwiler et al., 2005). The underlying mechanisms include nutrient transfer among litter types, complementary resource use, stimulatory or inhibitory influences of specific litter compounds, creation of a favorable micro-environment for decomposition and interactions across trophic levels (Hättenschwiler et al., 2005; Gessner et al., 2010). However, effects of litter mixtures from deciduous and evergreen trees on the decomposition process in subtropical karst forests remain unclear. There is little knowledge on litter decomposition rates of different plant species for this area (Wang and Xu, 2013). Given that leaf litter from deciduous species usually has a higher decomposition rate than that from evergreen species (Cornwell et al., 2008), the high richness of deciduous trees, to some extent, could accelerate the nutrient turnover of karst ecosystems even if mixing effects are additive. Non-additive effects on decomposition are also possible in karst forests because mixing litter from deciduous and evergreen species creates a blend of materials with diverse leaf structures, chemical characteristics and alters the micro-environment in which decomposition occurs (Hector et al., 2000; Schimel and Hättenschwiler, 2007). As fast-decomposing species with high litter quality usually accelerate decomposition rates of slow-decomposing species in litter mixtures through nutrient transfer (Hättenschwiler et al., 2005), we firstly hypothesized that leaf litter of deciduous trees would enhance the decomposition of evergreen leaf litter, leading to a positive non-additive effect.

Despite extensive testing in different ecosystems, the relationship between species diversity and litter decomposition remains controversial (Hättenschwiler et al., 2005; Gessner et al., 2010). Previous studies of litter mixture decomposition in temperate and tropical forests focused on mass loss and nitrogen (N) dynamics, ignoring the cycling of other essential nutrients (Hättenschwiler et al., 2005; Bonanomi et al., 2010). Subtropical karst forests support a remarkably high biodiversity (Zhu, 1993) despite their shallow topsoils, leading to the hypothesis that litter decomposition is important for recycling many essential nutrients so that they are bioavailable for the plant community, but this remains to be determined. We secondly expected that increasing litter species richness would strengthen the synergistic effects on litter decomposition. Moreover, recent studies suggested that incubation time significantly influences the mixing effects, and should be accounted for in the future (Lecerf et al., 2011; Montané et al., 2013; Wu et al., 2013). We also expected that non-additive effects would change with decomposition time in karst forests due to the temporal changes in litter quality, microclimate and dominant decomposers during the decomposition process.

In this study, we selected three deciduous and three evergreen trees with different litter qualities from a typical karst forest in southwestern China, to examine the mixing effects on mass loss and multiple nutrient dynamics of decomposing litter mixtures. Different litter mixing treatments with species numbers in the

range of 1–6 were designed to examine how litter species richness affected mixing effects.

2. Materials and methods

2.1. Study site

This study was carried out at Puding Karst Ecosystem Research Station, Chinese Academy of Sciences in Guizhou Province, China (26°16'1"N, 105°46'39"E). Long-term mean annual precipitation and temperature of this region are 1390 mm and 15.1 °C, respectively. Annual precipitation was 1399.5 mm in 2012 and 725.4 mm in 2013, about 70% of which fell during May–August (Fig. S1). Typical vegetation is mixed evergreen and deciduous broad-leaved forest, dominated by *Platycarya longipes*, *Quercus aliena*, *Lithocarpus confinis* and *Itea yunnanensis*. Common species also include *Carpinus pubescens*, *Celtis bungeana*, *Cinnamomum bodinieri*, *Machilus cavaleriei* and *Pittosporum brevicalyx*. *Platycarya longipes*, *Quercus aliena*, *Carpinus pubescens* and *Celtis bungeana* are deciduous trees and the others are evergreen. The deciduous trees shed their leaves every winter and are dormant during the dry period. The mean height of the tree canopy is about 15 m and mean canopy coverage is about 85%. The soil type is limestone soil, according to Chinese soil genetic classification, or similar to Rendoll according to USDA Soil Taxonomy (Soil Survey Staff, 1999). The topsoil in this region is extremely shallow, in the range of 0–30 cm deep. The soil pH in the study area was 6.8 and soil organic carbon (C) concentration was 77.36 g kg⁻¹. Nutrient concentrations in the soil were as follows (all g kg⁻¹): N 5.60, phosphorus (P) 0.68, potassium (K) 14.83, calcium (Ca) 12.07, iron (Fe) 36.40, magnesium (Mg) 9.86, manganese (Mn) 0.63 and sulfur (S) 1.12.

2.2. Plant material and experimental design

Six broad-leaved trees naturally occurring in the karst forests were selected: three deciduous (*C. pubescens*, *P. longipes* and *Q. aliena*) and three evergreen trees (*I. yunnanensis*, *L. confinis* and *M. cavaleriei*). At the beginning of December 2011 when most of the litter fall occurred, fresh and intact leaf litter was directly collected from the forest floor, air-dried for 10 d and stored for a week at room temperature (15–25 °C). The chemical characteristics of these litter types are described in Table 1.

An experiment was conducted using the litter bag method in the field. Each nylon litter bag (15 cm × 20 cm, 1 mm mesh) was filled with 10 g of air-dried leaf litter. Subsamples of initial litter were oven-dried (65 °C for 48 h) to calculate the correction factor for converting air-dried mass to water-free dry mass. This experiment included nine types of litterbags: (1) six monospecific types from each species; (2) a mixture of two species (a deciduous and an evergreen tree, *P. longipes* and *I. yunnanensis*); (3) a mixture of four species (two deciduous and two evergreen trees, *P. longipes*, *Q. aliena*, *I. yunnanensis* and *L. confinis*); and (4) a mixture of all the studied six species (three deciduous and three evergreen trees). The ratio of deciduous:evergreen species numbers was 1:1 in all mixtures. The priority of species selection in the mixtures was based on their dominance in karst forests. For example, *I. yunnanensis* was included in all three litter mixtures and *M. cavaleriei* only in the six-species mixture because *I. yunnanensis* is the most dominant evergreen species in the forest and constitutes the largest proportion of evergreen litter on the forest floor, and *M. cavaleriei* is the least dominant evergreen species. All mixtures were filled with individual litter species in equal proportions. On 23 December 2011, 288 litterbags (nine types × four replicates × eight sampling dates) were placed on the forest floor in four replicate blocks and retrieved every three months during the

Table 1
Initial qualities and final mass remaining of monospecific litter from six species after 720 d of decomposition (mean \pm SE).

	<i>Itea yunnanensis</i>	<i>Lithocarpus confinis</i>	<i>Machilus cavaleriei</i>	<i>Carpinus pubescens</i>	<i>Platycarya longipes</i>	<i>Quercus aliena</i>
Lignin (%)	27.16 \pm 1.47 b	23.17 \pm 1.64 ab	25.06 \pm 0.70 ab	20.17 \pm 0.59 a	26.96 \pm 2.91 ab	22.64 \pm 1.40 ab
C (g kg ⁻¹)	439.36 \pm 3.30bc	453.00 \pm 6.31 c	484.42 \pm 6.30 d	418.68 \pm 3.88 b	393.49 \pm 4.66 a	417.52 \pm 6.33 b
Ca (g kg ⁻¹)	29.49 \pm 1.32 c	18.85 \pm 0.97 b	12.92 \pm 0.40 a	33.98 \pm 0.52 d	37.73 \pm 0.94 d	27.26 \pm 0.90 c
Fe (g kg ⁻¹)	0.28 \pm 0.03 a	0.23 \pm 0.03 a	0.26 \pm 0.02 a	0.63 \pm 0.04 b	0.23 \pm 0.03 a	0.26 \pm 0.03 a
K (g kg ⁻¹)	1.68 \pm 0.13 b	1.62 \pm 0.11 b	0.83 \pm 0.02 a	1.82 \pm 0.12 bc	2.21 \pm 0.03 c	4.34 \pm 0.49 d
Mg (g kg ⁻¹)	1.52 \pm 0.01 a	2.28 \pm 0.07 bc	1.22 \pm 0.05 a	2.64 \pm 0.02 d	2.09 \pm 0.04 b	2.49 \pm 0.12 cd
Mn (g kg ⁻¹)	0.12 \pm 0.01 c	0.24 \pm 0.02 d	0.07 \pm 0.01 a	0.13 \pm 0.01 c	0.09 \pm 0.00 b	1.74 \pm 0.06 e
N (g kg ⁻¹)	12.54 \pm 0.51 bc	14.60 \pm 0.80 cd	11.72 \pm 0.36 ab	12.41 \pm 0.22 abc	15.08 \pm 0.30 d	10.23 \pm 0.60 a
P (g kg ⁻¹)	0.55 \pm 0.08 d	0.48 \pm 0.03 d	0.40 \pm 0.02 cd	0.31 \pm 0.02 bc	0.29 \pm 0.02 b	0.17 \pm 0.01 a
S (g kg ⁻¹)	1.96 \pm 0.06 bc	1.67 \pm 0.07 ab	1.37 \pm 0.03 a	2.26 \pm 0.10 cd	2.28 \pm 0.09 d	1.70 \pm 0.02 b
C: N	35.28 \pm 1.44 bc	31.51 \pm 2.24 ab	41.51 \pm 1.57 c	33.78 \pm 0.53 b	26.12 \pm 0.38 a	41.21 \pm 1.78 c
C: P	850.2 \pm 100.5 a	972.6 \pm 87.2 ab	1215.7 \pm 58.0 ab	1381.4 \pm 71.7 b	1392.7 \pm 84.6 b	2529.4 \pm 170.3 c
Lignin: N	21.70 \pm 0.92 ab	16.02 \pm 1.23 a	21.51 \pm 1.13 ab	16.29 \pm 0.65 a	17.82 \pm 1.79 ab	22.53 \pm 2.12 b
Mass remaining (%)	38.63 \pm 6.14 ab	30.71 \pm 2.34 ab	48.22 \pm 3.88 b	37.01 \pm 3.57 ab	28.03 \pm 3.85 a	44.12 \pm 3.96 ab

I. yunnanensis, *L. confinis* and *M. cavaleriei* are evergreen trees, while *C. pubescens*, *P. longipes* and *Q. aliena* are deciduous trees. Different letters within the same rows indicate significantly different means ($p < 0.05$).

following two years. Fresh litter was allowed to fall onto the litter bags during the experiment. On each sampling date, litterbags were transported to the laboratory and the remaining litter was brushed carefully to remove adhering soil particles, roots and fauna. We tried to separate all mixtures into individual litter species to identify the species-specific litter mass remaining. However, only samples collected at the first two times could be clearly separated. All samples were oven-dried (65 °C for 48 h) to determine litter mass and stored in sealed plastic bags for 10–15 d at room temperature for further chemical analyses.

2.3. Chemical analyses

Total N concentration was analyzed using the Kjeldahl method and concentrations of other nutrients (Ca, Fe, K, Mg, Mn, P and S) were determined with an inductive coupled plasma emission spectrometer after digestion of samples in concentrated nitric acid. Total C was determined using the oil-bath potassium dichromate titration method (Dong et al., 1996). Acid-indigestible fiber was determined using the method of Van Soest (1963) and is hereafter referred to as lignin.

2.4. Statistical analyses

Litter mass remaining was calculated in percentage of initial litter dry mass at each sampling date. Nutrients and lignin remaining were also calculated as percentages of their respective amounts in the initial litter mass. Expected mass remaining of each litter mixture was calculated as the sum of each monospecific litter's mass remaining, weighted by its proportion in the mixture. Expected nutrients and lignin remaining were calculated in a similar way. Decomposition constants (k) of the six single-species litter bags were calculated using mass remaining data (Olson, 1963) as: $W_t = W_0 e^{-kt}$, where W_0 is the initial litter mass and W_t is the litter mass remaining at time t (years). The k -values were derived as the slope of a linear regression of $\log_e(W_t/W_0)$. According to Wardle et al. (1997), the relative mixing effect was calculated for each mixture as: [(expected – observed)/expected] \times 100 remaining. The values differing from zero indicate non-additive effects on decomposition. Positive and negative deviations from zero imply synergistic (acceleration of decomposition) and antagonistic (deceleration of decomposition) effects, respectively.

Data on litter mass, lignin and nutrients were subjected to repeated measures ANOVA to determine differences among six litter species and among three mixtures (two, four and six species) across the whole decomposition period, with time as a within-

subject effect and litter species or mixture as between-subject effects. Student's t -tests were performed to test whether synergistic or antagonistic effects in litter mixtures significantly differed from zero at each sampling time. Repeated measures ANOVA was also used to test the differences of relative mixing effects among three mixtures, with time as a within-subject effect. At the first two sampling dates, data on individual litter species of *I. yunnanensis*, *L. confinis*, *P. longipes* and *Q. aliena* in the litter mixtures were subjected to one-way ANOVA and data on *C. pubescens* and *M. cavaleriei* were subjected to t -tests to determine differences among litter species richness treatments. Pearson's correlation was used to assess the relationships between initial litter qualities and litter mass loss, lignin or nutrient remaining in the single-species litter bags. Data were log-transformed to satisfy the assumption of normality. All effects were considered significant at $p < 0.05$. Statistical analyses were performed using SPSS version 17.0 (SPSS, USA).

3. Results

3.1. Decomposition of monospecific litter

Initial chemical characteristics varied significantly among the six litter species, with no clear pattern between deciduous and evergreen species (Table 1). The *P. longipes* had the highest Ca, N and S concentrations, while *C. pubescens* had the highest Fe and Mg concentrations. The K and Mn concentrations were highest in *Q. aliena*, while concentrations of P and lignin were highest in *I. yunnanensis*. Due to higher N concentrations and lower ratios of C:N and lignin:N, *P. longipes* and *L. confinis* were determined to have higher litter qualities than the other four species.

After 720 d of decomposition, litter mass remaining of the six species in single-species litter bags increased in the following order: *P. longipes* (28.0%) < *L. confinis* (30.7%) < *C. pubescens* (37.0%) < *I. yunnanensis* (38.6%) < *Q. aliena* (44.1%) < *M. cavaleriei* (48.2%) (Table 1). The corresponding k -values for the six species were 0.642, 0.613, 0.470, 0.491, 0.427 and 0.322 year⁻¹. Only *P. longipes* litter decomposed significantly faster than *M. cavaleriei* litter. Across the experimental period, all six species revealed higher decomposition rates during the first year, except for *M. cavaleriei* which showed an initially relatively stable and low decay rate and more rapid decomposition at the end of the experiment (Fig. 1).

During decomposition, concentrations of Ca, Fe, Mn, N, P and S gradually increased in all six species, except for *I. yunnanensis* with a relatively stable P concentration (Fig. S2). The *Q. aliena* always

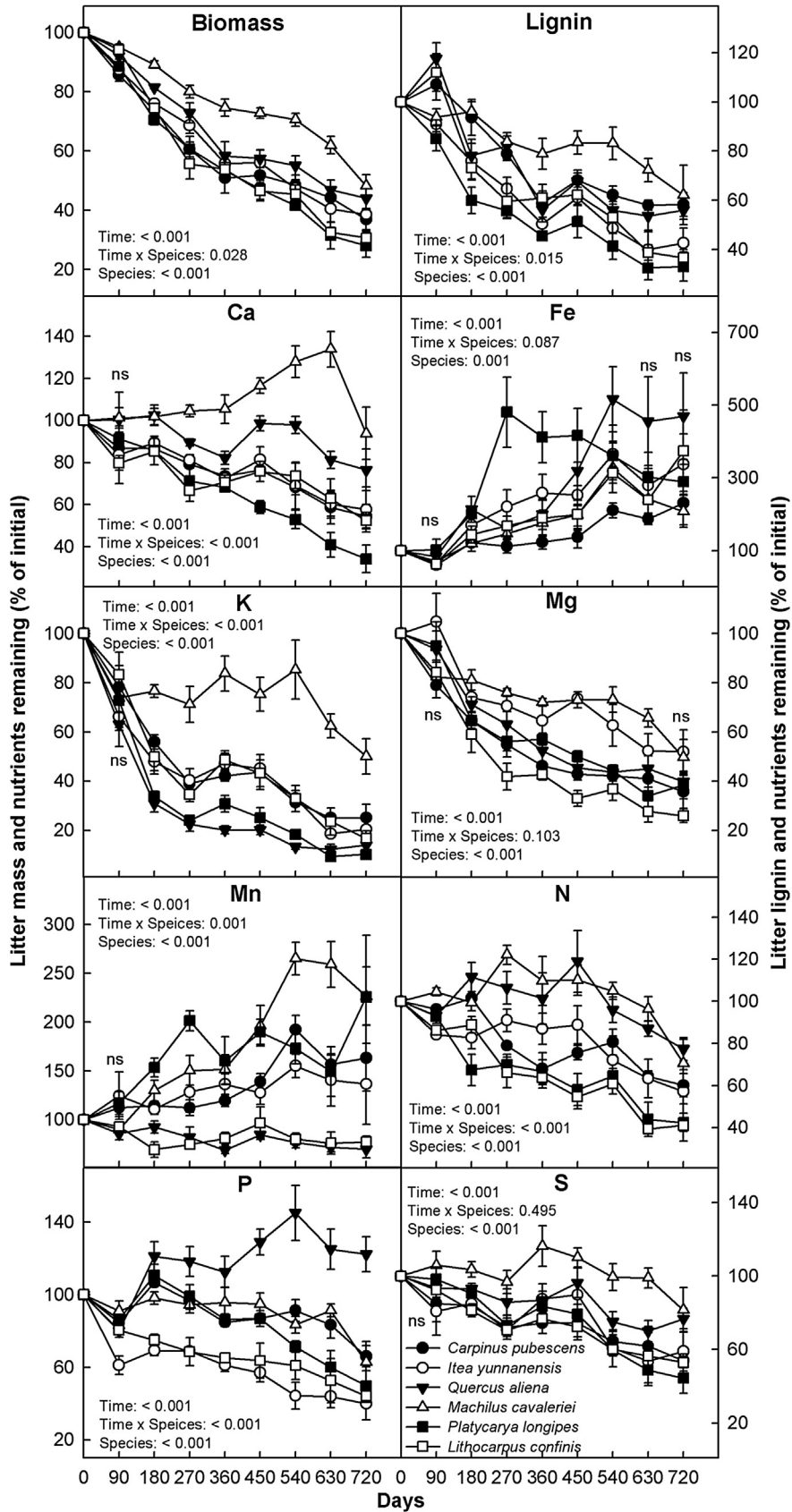


Fig. 1. Litter mass, lignin and nutrients remaining in single-species litter bags during the decomposition process, expressed as a percentage of initial values (mean ± SE; n = 4). Significant differences among litter species were observed at most sampling dates. ns, no significant difference among litter species.

showed a significantly higher Mn concentration than the other five species. However, K concentrations significantly declined in most species, especially during the early stage. Different litter species showed different changes of Mg concentration during decomposition.

The dynamics of total lignin and nutrient amounts depended on litter species and time (Fig. 1). Lignin reductions in the six species roughly followed the patterns of litter mass loss. Generally, Fe and Mn showed strong immobilization, while progressive releases of Ca, K, Mg, N and S occurred in most species. The Ca in *M. cavaleriei* and N in *Q. aliena* and *M. cavaleriei* also showed transient immobilization. The dynamics of P differed greatly among species during decomposition.

When pooling all data on single-species litter bags together, final litter mass loss was positively correlated with initial N concentrations and negatively with initial C:N ratios ($p < 0.05$; Table 2). The values of lignin, N and S remaining were negatively correlated with initial N concentrations and positively with initial C:N ratios. Final Ca remaining was negatively correlated with initial S concentrations and positively with initial C:N ratios. There was a positive relationship between final P remaining and initial K and Mn concentrations, and a negative relationship between P remaining and initial P concentrations.

3.2. Decomposition of litter mixtures

In terms of litter mass, lignin and nutrients, decomposition rates were usually highest in the four-species, lowest in the six-species and intermediate in the two-species mixture at most sampling dates (Fig. 2). All three mixtures showed significant Fe immobilization, with small differences between mixtures. High Mn immobilization in the two-species mixture was observed in the middle period of decomposition, while slow Mn releases occurred in the four- and six-species mixtures over time. After 2 years, values of litter mass, lignin and nutrients remaining in the two-species mixture were close to those of the faster single-species litter of the two component species (*P. longipes* and *I. yunnanensis*) (Figs. 1 and 2). In the four- and six-species mixtures, decay rates of all measured variables were not higher than the highest values or lower than the lowest values of the monospecific litter types.

3.3. Synergism and antagonism in litter mixtures

Considering data from all mixture treatments over time, litter mixing caused synergistic effects on litter mass and nutrients remaining but not lignin remaining (Fig. S3). For example, 65.6% of all mixtures showed lower litter mass remaining than expected, leading to an overall 5.0% synergistic effect (i.e., litter mixing accelerated mass loss by 5.0%). The overall synergistic effect on

nutrients remaining increased in the following order: S (4.8%) < Ca (5.2%) < N (6.4%) < Mg (6.5%) < K (11.1%) < P (14.8%) < Fe (24.8%) < Mn (26.2%).

Non-additive effects were also related to mixture treatment and incubation time (Fig. 3). Overall synergistic effects in the four-species mixture were observed for all measured variables, ranging from 12.1% for S to 35.3% for Fe; while those in the two- and six-species mixtures, respectively, for Fe were 21.6 and 17.3%, for Mn were 12.5 and 32.1%, and for P were 10.0 and 9.5%. Moreover, with regard to litter mass, lignin, Mn and P, the overall synergistic effects were stronger in the four-species than in the other mixtures. Mixing effects in the three mixtures showed different dynamic patterns with time. Most non-additive effects occurred after 180 d of decomposition and were synergistic (Fig. 3). Antagonistic effects were occasionally observed in the two-species (three times) and six-species (once) mixtures. Synergistic effects on litter mass, lignin and nutrients in the four-species mixture generally increased over time, with a small depression in the middle period. At the end of experiment, the synergistic effects in the four-species mixture were 27.1% for lignin, 28.4% for Ca, 30.0% for litter mass, 31.3% for N, 35.2% for Mg, 45.4% for P, 56.6% for Fe and 64.9% for Mn, and the largest effects on K (32.9%) and S (30.4%) were observed after 630 d. However, mixing effects in the two- and six-species mixtures showed large fluctuations. Significant non-additive effects in the two mixture types were mainly observed at 180, 270, 630 and 720 d and there was no significant accelerating or decelerating effect on lignin.

3.4. Effect of litter species richness on individual litter species

After 180 d of decomposition, as litter species richness increased, *I. yunnanensis* litter decayed significantly faster in the six-species mixture than in the single-species litter bags, with respect to litter mass, Ca, Fe, P and S (Fig. 4). In contrast, slower decomposition of *P. longipes* litter was observed with increasing species numbers based on numerically higher values of litter mass, lignin, K and Mg, as well as significantly greater P concentration in multi-species mixtures. Moreover, Mn remaining in *P. longipes* increased significantly with species numbers up to four and then decreased in the six-species mixture. As litter species number increased, mass and lignin remaining in *L. confinis* litter increased significantly in the six-species mixture, while N remaining decreased in four- and six-species mixtures. Being mixed with the other five species accelerated decomposition of *M. cavaleriei* litter, with regard to litter mass, lignin, Mg and S. Litter mixing did not significantly affect decomposition of *C. pubescens* and *Q. aliena* litter, except that Fe remaining in *C. pubescens* decreased significantly in the six-species mixture.

Table 2
Summary of Pearson's correlation coefficients (*R*) relating litter mass loss and lignin and nutrients remaining after 720 d of decomposition to initial litter qualities in the six single-species litter bags.

	Initial lignin	Initial Ca	Initial Fe	Initial K	Initial Mg	Initial Mn	Initial N	Initial P	Initial S	Initial C:N	Initial lignin:N
Mass loss	0.14	0.55	-0.04	-0.07	0.41	-0.36	0.90 *	0.13	0.66	-0.97 **	-0.76 ^a
Lignin	-0.52	-0.36	0.47	0.04	0.07	0.28	-0.84 *	-0.32	-0.40	0.83 *	0.40
Ca	-0.16	-0.75 ^a	-0.10	-0.02	-0.44	0.33	-0.79 ^a	-0.07	-0.83 *	0.96 **	0.66
Fe	-0.05	0.06	-0.44	0.80 ^a	0.39	0.80 ^a	-0.23	-0.24	-0.15	0.14	0.29
K	-0.04	-0.69	0.13	-0.62	-0.64	-0.34	-0.36	0.25	-0.60	0.60	0.27
Mg	0.55	-0.09	-0.12	-0.18	-0.76 ^a	-0.09	-0.47	0.22	-0.18	0.48	0.79 ^a
Mn	0.41	0.12	0.09	-0.56	-0.51	-0.64	0.26	0.03	0.20	-0.22	-0.04
N	-0.28	-0.28	0.15	0.38	-0.12	0.60	-0.98 **	-0.42	0.44	0.92 **	0.75 ^a
P	-0.46	-0.01	0.05	0.83 *	0.42	0.92 **	-0.77 ^a	-0.82 *	-0.23	0.61	0.47
S	-0.11	-0.66	-0.18	0.16	-0.39	0.48	-0.85 *	-0.19	-0.80 ^a	0.96 **	0.76 ^a

* $p < 0.05$; ** $p < 0.01$; ^a $p < 0.1$. Means of four replicates per species were used in the correlation analysis.

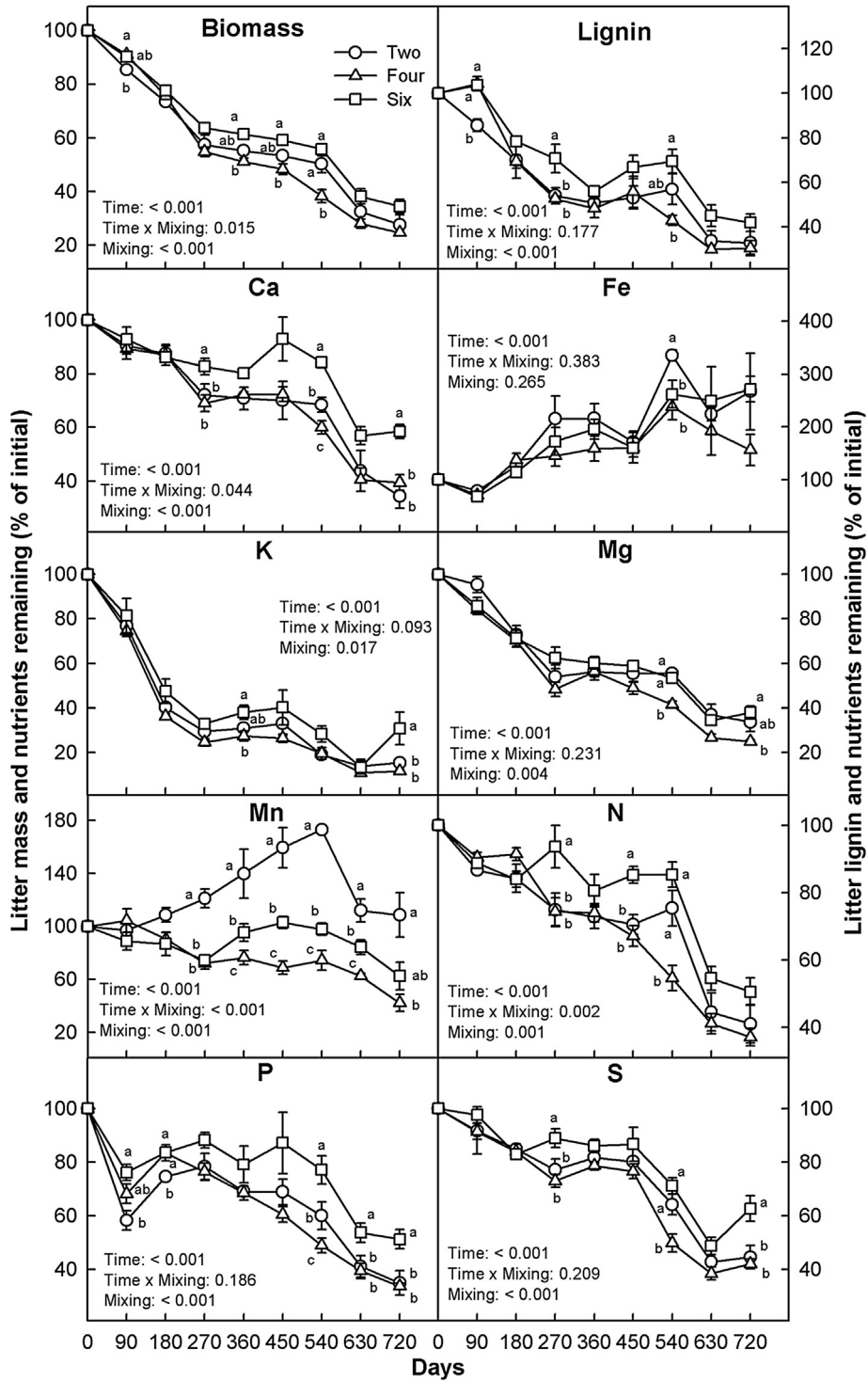


Fig. 2. Litter mass, lignin and nutrients remaining of the three mixtures with different species richness levels during the decomposition process, expressed as a percentage of initial values (mean \pm SE; n = 4). Three mixtures: two-species (*I. yunnanensis* and *P. longipes*), four-species (*I. yunnanensis*, *L. confinis*, *P. longipes* and *Q. aliena*) and six-species mixtures (*C. pubescens*, *I. yunnanensis*, *L. confinis*, *M. cavaleriei*, *P. longipes* and *Q. aliena*). The ratio of deciduous to evergreen species numbers was 1:1 in all mixtures. Different letters indicate significant differences among species richness levels at $p < 0.05$ by LSD tests.

4. Discussion

4.1. Decomposition of monospecific litter

The k-values for all investigated species (0.494 year^{-1}) in the present study were close to the 0.574 year^{-1} calculated from 96

plant species in the same region by Wang and Xu (2013). However, the mean k-values in the karst region were lower than the average of 0.98 year^{-1} reported for Chinese plants (Huang et al., 2010) and means of $1.048\text{--}1.726 \text{ year}^{-1}$ for dominant species in subtropical evergreen broad-leaved forests in non-karst regions in the same latitudinal zone (Wang et al., 2004; Li et al., 2011). As also reported

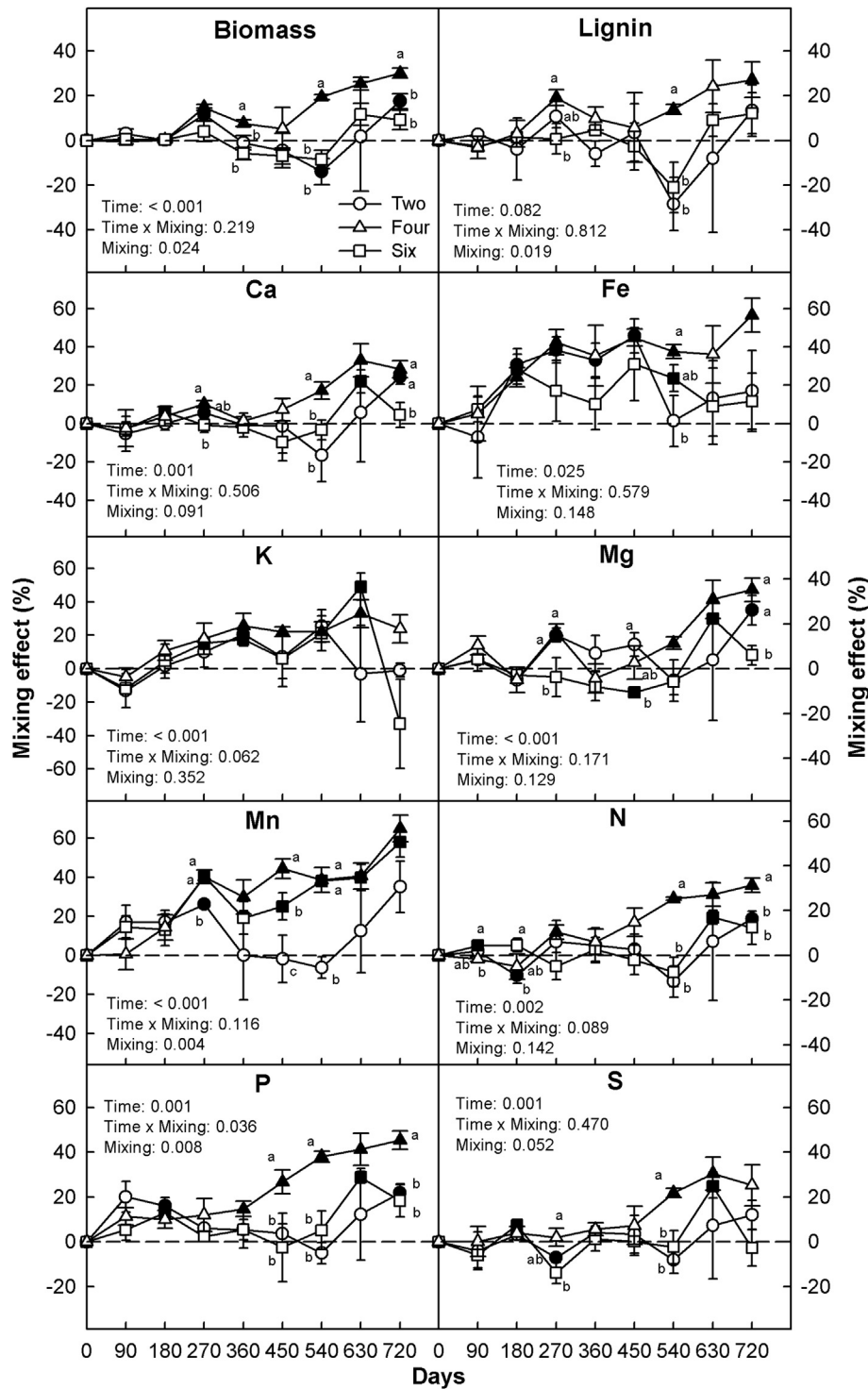


Fig. 3. Mean relative litter mixing effects in the three mixtures with different species richness levels during the decomposition process (mean \pm SE; $n = 4$). Three mixtures: two-, four- and six-species mixtures. The ratio of deciduous to evergreen species numbers was 1:1 in all mixtures. Closed symbols represent significant non-additive effects. Positive deviations from zero indicate synergistic effects and negative deviations indicate antagonistic effects. Different letters indicate significant differences among species richness levels at $p < 0.05$ by LSD tests.

elsewhere (Cornwell et al., 2008; Bonanomi et al., 2010; Jacob et al., 2010; Wu et al., 2013), decomposition rates of karst plant species largely depended on initial nutrient status, especially N concentrations and C:N ratios (Table 2). Therefore, the very low decomposition rates could be mainly explained by the low leaf nutrient concentrations resulting from the limited soil nutrient availabilities

in such harsh habitats (Wang and Xu, 2013; Liu et al., 2014). For instance, total N stock in the topsoil of a karst forest (4.29 t ha^{-1} ; Du et al., 2010) was remarkably lower than that of the subtropical evergreen broad-leaved forests in a non-karst region in the same latitudinal zone (12.13 t ha^{-1} ; Yan et al., 2007). Moreover, in the present study, there were higher decomposition rates during

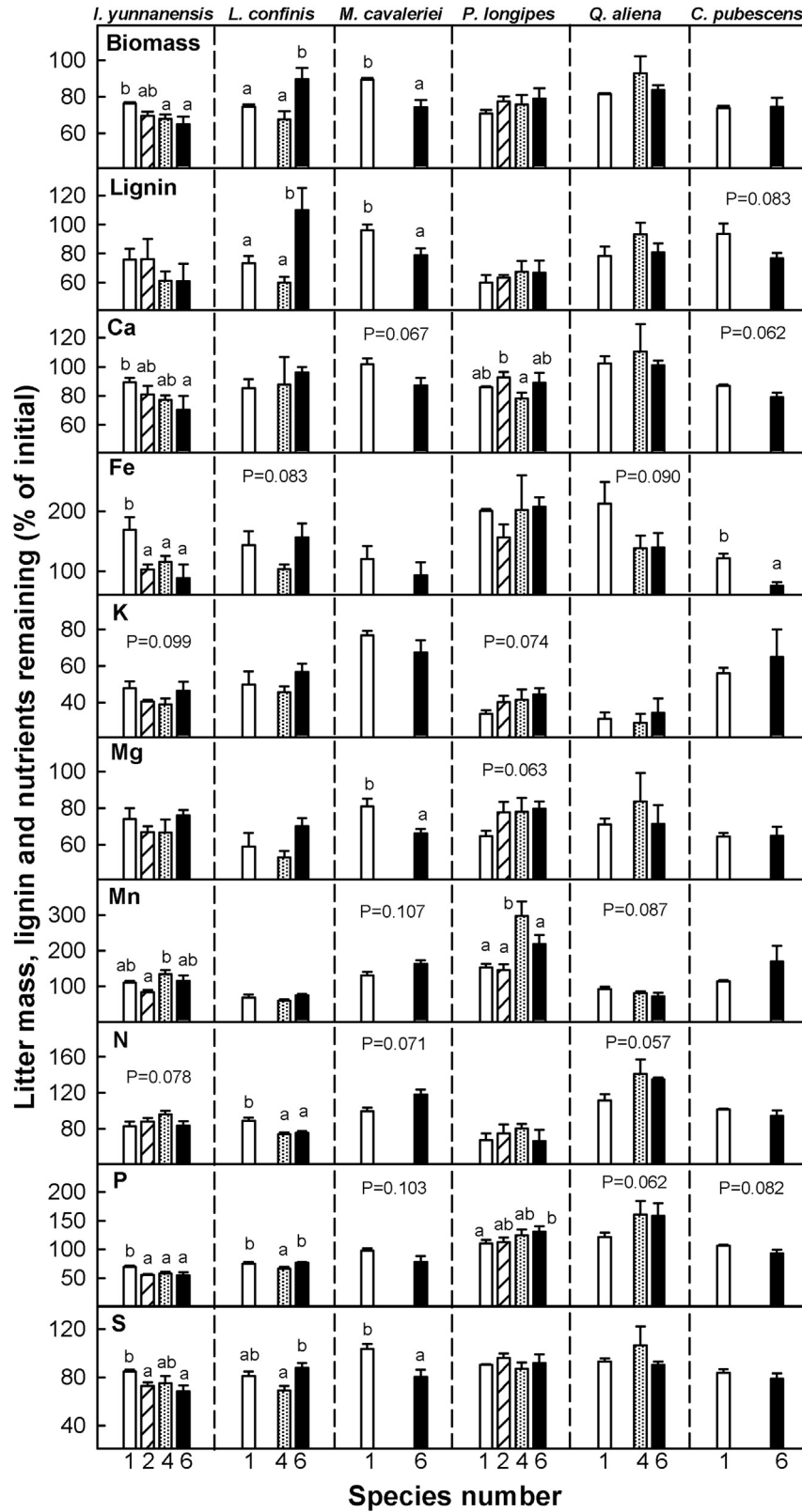


Fig. 4. Litter mass, lignin and nutrients remaining of individual litter species after 180 d of decomposition (mean \pm SE; n = 4). Litter was mixed at different species richness levels: single-species litter (1) and two-species (2), four-species (4) and six-species mixtures (6). Different letters indicate significant differences among species richness levels at $p < 0.05$ by LSD tests or t -tests.

summer and autumn when rainfall was more abundant and temperatures were higher (Figs. 1 and S1), especially for *P. longipes* and *L. confinis*, which showed the highest initial N concentrations but

low decay rates during the first 90 d in winter. This indicated that climate was also an important factor controlling decomposition in karst ecosystems. In contrast to other studies, decomposition rates

did not significantly correlate with initial lignin concentrations and lignin:N ratios (Table 2), which may be a result of the small range of lignin concentrations and the limited incubation time for lignin to have an effect (Berg and McClaugherty, 2008; Jacob et al., 2010).

At the plant functional group level, there was no overall significant difference in decomposition between evergreen and deciduous trees, with the exception that deciduous trees released K faster and P more slowly than evergreen trees. This was in contrast to a global report that litter from deciduous species decomposed 60% faster than that from evergreen species (Cornwell et al., 2008). The studied deciduous trees did not have significantly higher litter quality (e.g., higher initial N concentrations and lower C:N ratios) than the evergreen trees (Table 1). Our observations did not support the expectation that deciduous trees could accelerate nutrient cycling of karst forests without non-additive effects, due to their faster decomposition rates than evergreen trees.

4.2. Effect of litter richness on litter mixtures

Considering all mixtures over time, there was a strong synergistic effect on mass loss and nutrient release in the karst forest (Fig. S3), which emphasized the prevailing positive interactions in litter mixtures (Gartner and Cardon, 2004; De Marco et al., 2011). However, based on data collected at each sampling date, synergistic effects only accounted for 31.3% of all cases (71 out of 240), and mainly occurred in the four-species mixture. Positive effects on decomposition of the whole mixture were usually stronger in four- than in the two- and six-species mixtures (Fig. 3). At individual species level, the studied species also showed idiosyncratic responses to litter species richness (Fig. 4). This did not support our second hypothesis that synergistic effects would increase with litter species richness in karst ecosystems. Litter mixing effects could be driven by the extent to which the combination of complementary resources from litter species meets the requirements of the different components of the decomposer community (Vos et al., 2013). Our results were influenced by the species selection at each litter richness level. Both two- and four-species mixtures had an even combination of litter species with contrasting N concentrations. As decomposition was mainly limited by N in the karst habitats, *I. yunnanensis* and *Q. aliena* in the mixtures could profit from their N-rich neighbors, which may have enhanced the positive effects. The high initial Mn concentration of *Q. aliena* litter increased the Mn level of the four-species mixture, which could enhance lignin degradation (Berg et al., 2010). However, the addition of two species (*C. pubescens* and *M. cavaleriei*) with low N concentrations to the six-species mixture did not increase the N level of the source leaf litter transferring N nor did they enhance the N gradient among litter species. If N transfer among different litter species is important for synergistic effects, the former would control the available N concentration and the latter would determine the rate of N transfer (Schimel and Hättenschwiler, 2007). If the species combinations were changed, non-additive effects would show a different pattern as species richness increased. For example, if the two-species mixture was two species with low quality (*Q. aliena* and *M. cavaleriei*), the four-species mixture included one species with a high N concentration, and the six-species mixture contained more N-rich species, we would expect an increasingly positive mixing effect on decomposition. Further research including more species combinations of different plant functional types at each species richness level is needed to elucidate how species composition and species richness influence litter mixing effects on decomposition in karst forests.

Interestingly, regardless of the initial litter quality and decomposition rate of component species, mixing effects were larger for evergreen than deciduous species. For example, only two evergreen

species (*I. yunnanensis* and *M. cavaleriei*) showed increased mass loss in the six-species mixture after 180 d of decomposition (Fig. 4). This supported our first hypothesis that leaf litter of deciduous trees would promote the decomposition of litter from evergreen trees, leading to a positive non-additive effect. Previous studies have demonstrated that nutrient transfer was an important mechanism for synergistic effects in litter mixtures (Schimel and Hättenschwiler, 2007; Montané et al., 2013). In our study, only *P. longipes* litter resulted in a significant increase of Mn in the four-species mixture and P in the six-species mixture (Fig. 4). The N transport to *I. yunnanensis*, *M. cavaleriei* and *Q. aliena* litter from their richer neighbors in the mixtures was not significant at the early decomposition stage ($p < 0.1$). Obvious nutrient transfer could occur later when the mixing effects were stronger in the late decomposition stage. Additionally, Barantal et al. (2011) argued that decomposition of recalcitrant litter species may be accelerated in litter mixtures because inhibitory compounds are less concentrated, allowing a more abundant decomposer community per unit mixed litter mass. This kind of dilution effect may have led to the faster decay of *I. yunnanensis* and *M. cavaleriei* (with high initial lignin concentrations) in the six-species mixture. Furthermore, the positive interactions could be partly explained by the changes of micro-environment. We previously observed that litter from deciduous trees was more efficient in water absorption than that from evergreen trees, while the latter had a higher capacity to prevent water evaporation in this ecosystem (Liu et al., 2011b). As frequent temporary drought, resulting from the shallow and patchy soil and the high porosity of limestone, is one of the most important limiting factors in karst ecosystems (Liu et al., 2010), the combination of the two litter types could contribute to maintaining a favorable and stable moisture condition for litter decomposition. Similar litter mixing effects on the micro-environment have been reported for other ecosystems (Hector et al., 2000; Hoorens et al., 2002, 2010). A more diverse litter layer with different leaf sizes, leaf shapes and leaf-surface structures also created a more diverse microhabitat structure than a monospecific litter layer for different types of soil animals and microorganisms (Hättenschwiler et al., 2005).

Litter decomposition is a fundamental process of biogeochemical cycles and a critical determinant of nutrient turnover (Vitousek, 1982). Currently, how biodiversity affects decomposition remains controversial (Hättenschwiler et al., 2005; Gessner et al., 2010). Many studies have revealed that species composition is more important than litter species richness in driving non-additive effects on decomposition in tropical (Hättenschwiler and Jørgensen, 2010; Barantal et al., 2011) and temperate forests (Vivanco and Austin, 2008; Jacob et al., 2010; Wu et al., 2013), whereas little is known about subtropical forests. Recently, Trogisch et al. (2016) reported that litter species richness marginally affected non-additive effects across 27 subtropical forest stands in China. Our current study provides further evidence that species composition has a larger influence than species richness on the strength of non-additive effects on decomposition in subtropical forests.

Consistent with several studies (De Marco et al., 2011; Lecerf et al., 2011; Montané et al., 2013; Wu et al., 2013) and our expectation, our results emphasized that non-additive effects varied greatly with time, which could result from changes of microclimate, litter chemical components and decomposer community (Srivastava et al., 2009; Montané et al., 2013; Wu et al., 2013). Synergistic effects became stronger with prolonged decomposition time, especially in the four-species mixture. One possible explanation is the successional change in dominant litter consumers (fungi, detritivores and bacteria) that synergistically participate in decomposing litter (Lecerf et al., 2005; Gessner et al., 2010). The later dominant colonizers, such as large detritivores, are more likely

to cause stronger non-additive effects on decomposition than the early dominant colonizers, such as fungi (Schindler and Gessner, 2009; Lecerf et al., 2011). In addition, stronger non-additive effects occurred in the rainy season than in the drought season, suggesting that favorable wet conditions, to some extent, were a trigger for non-additive effects in this water-limited ecosystem. Unfortunately, our current study could not address a comprehensive explanation for the different patterns of non-additive effects over time between the three mixture types.

5. Conclusions

The decomposition of leaf litter in a karst forest was mainly controlled by N concentration of the litter mixture. Litter mixing caused synergistic effects on mass loss and nutrient release. Species composition was more important than species richness in driving non-additive effects on decomposition in subtropical karst forest. Deciduous trees in karst ecosystems significantly contribute to nutrient cycling by promoting decomposition of evergreen leaf litter. Karst trees growing in a limited soil volume show significantly lower concentrations of multiple nutrients (e.g., N, P, K, S, Fe and Mn) than Chinese plants generally (Liu et al., 2014), suggesting that they could be limited by multiple nutrients other than N or P (Zhang and Wang, 2009). The recycling of essential elements through litter decomposition seems to be particularly important for the nutrient budget of karst ecosystems. High tree species richness significantly accelerated nutrient release from leaf litter, which could in turn contribute to supporting high biodiversity in the karst forest in such harsh habitats.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2016.07.004>.

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