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Functional evenness of N-to-P ratios of evergreen-deciduous mixtures predicts positive non-additive effect on leaf litter decomposition

Chao Guo · J. Hans C. Cornelissen · Qing-Qing Zhang · En-Rong Yan ⓘ

Abstract

Aims The effects of litter diversity on litter decomposition remain debated. We tested to what extent the community-weighted means (CWM; functional composition) versus Rao's dissimilarity of litter nitrogen (N)-to-phosphorus (P) ratios explain the non-additive mixture effect on decomposition rate (k) and associated N release.

Methods We carried out a one-year field decomposition experiment with a range of five litter types ranging from three evergreens only (high N/P and low specific leaf area, SLA) to three deciduous species only (low-N/P and high-SLA), with 30:70, 50:50 and 70:30% mixtures of these two extremes in between, in subtropical forest of China.

Results There were tight hump-backed relationships of absolute k-values and N release, respectively, with the CWM_{SLA}. The mixtures with the highest functional evenness in terms of $CWM_{N/P}$ caused the highest positive non-additivity on decomposition ($R^2 = 0.72$) and N release ($R^2 = 0.95$) rates. In contrast, the mixing effect on k or N release was weakly positively correlated with Rao's dissimilarity of N/P ($R^2 = 0.38$ and 0.27 respectively).

Conclusions Our results provide a strong framework for predicting litter decomposition rates and associated N release versus immobilization in mixtures of deciduous versus evergreen species based on their differences in initial stoichiometry.

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Introduction

Plant functional composition and diversity are important drivers of ecosystem processes (Adler et al. 2011; Fridley et al. 2012), they play a key role in ecosystem resource dynamics, particularly in carbon fluxes and nutrient cycling through the litter decomposition process in terrestrial ecosystems (Tilman et al. 2006). Plant trait-based approaches can be useful to understand the effect of changes in functional composition variation in litter quality via "afterlife" effects of green leaf traits

(Cornelissen 1996; Handa et al. 2014). However, how trait composition determines the decomposition of litter mixtures, directly or by interacting with litter from other species, remains debated.

Within an ecosystem, litter quality is the most important factor in controlling the efficiency of nutrient release and cycling processes of decomposition, with litter higher in nutrients and low in recalcitrant carbon-based compounds such as lignin is generally decomposed faster (Aerts 1997; Giller et al. 1997). However, litters of different species may also interact when in mixture. Based on nutrient transfer theory, summarized by (Hättenschwiler et al. 2005), the relatively nutrient-rich litter types could transfer nutrients to lower quality litter types, leading to more rapid decomposition of low-quality litter in the mixture. Experimental evidence supported that, more generally, litter mixing may have a non-additive effect on decomposition rate and nitrogen dynamics as the result of nutrient transfer between species (Gartner and Cardon 2004). Several studies have demonstrated that mixing litter can accelerate decomposition (Hector et al. 2000; Gartner and Cardon 2004; Handa et al. 2014). However, negative or lack of non-additivity on decomposition has also been reported repeatedly (Hoorens et al. 2003; Wardle et al. 2006). Positive non-additivity had a positive association (Barantal et al. 2011) or no association (Prescott et al. 2000) with leaf litter species composition and diversity. In addition, irrespective of mass loss, litter diversity may also have an effect on nitrogen (N) mineralization or immobilization during decomposition (Finzi and Canham 1998; Gartner and Cardon 2004). In order to work towards a predictive framework, trait-based approaches have been used to offer a tool for inferring mixing effects on decomposition processes based on litter composition and diversity. However, meaningful predictive power of green leaf or litter traits on the direction and size of the litter mixing effect on decomposition has been found in some (Hector et al. 2000; Gartner and Cardon 2004; Hättenschwiler and Gasser 2005; Handa et al. 2014) but not in other studies (Hoorens et al. 2010) and general relationships have still not been demonstrated. The lack of generality may partly be because some these studies have focused on variation in nutrient concentrations while other studies have focused on variation in nutrient stoichiometry. Depending on which is the limiting nutrient for biological processes in an ecosystem, this nutrient may be immobilized rather than mineralized by microbial decomposers. At ratios of nitrogen (N) and phosphorus (P) around 15 (the Redfield ratio) biological processes tend to be co-limited by N and P, while above this ratio P becomes more and more limiting and below this ratio N becomes more limiting (Koerselman and Meuleman 1996; Güsewell 2004). Here we propose that large differences in N/P ratio (rather than nutrient concentrations per se) between litter types also lead to more exchange of N and P between litters (via fungi) to alleviate the overall nutrient limitation of the litters and the fungi that colonize them (see above). Distinguishing between overall nutrient concentrations and release on the one hand and litter stoichiometry on the other hand, will improve our understanding of decomposition processes of litter mixtures in general, and those of deciduous-evergreen mixtures in particular.

Leaf litter of deciduous species generally has favourable structure and chemistry as usually indicated by high specific leaf area (SLA), low dry matter content and lignin concentration and (in some cases) high nutrient and labile carbon concentrations; these traits are the legacy of the resource acquisitive strategy of the living leaves. In contrast, green leaves and, consequently, leaf litter of evergreen species are high in recalcitrant structure with low SLA, high dry matter content and lignin and in some cases low nutrient concentrations. As a consequence of these trait differences, deciduous litter is generally broken down faster than evergreen litter (Cornelissen 1996; Wright et al. 2004; Cornwell et al. 2008; Reich 2014). However, deciduous leaves and litter also tend to have a lower N/P ratio than those of evergreens (e.g. deciduous 13.2, evergreen 20.1 in Wright et al. 2004; Han et al. 2005; Kang et al. 2010) and this is certainly the case for subtropical broadleaved forest of China, where N/P ratios of deciduous species can be much below the Redfield ratio (e.g. deciduous 11.5, evergreen 18.6 in Yan et al. 2010). If nutrient availability is a rate-limiting factor, then relatively nutritious deciduous species in litter mixtures may promote faster litter decay compared to mixtures without deciduous litter species (Gartner and Cardon 2004; Liu et al. 2016). However, in mixtures where low N limits the efficiency of microbial decomposer in respiring deciduous litter, evergreens might provide N during decomposition to help the microbes to break down the deciduous species faster during litter decomposition (Schimel and Hattenschwiler 2007; Handa et al. 2014). Thus, consistent with niche complementarity, we expect positive non-additive interactions between deciduous

and evergreen litter on decomposition based on differences both in overall decomposability (deciduous species promoting decomposition of evergreens) and in nutrient stoichiometry (evergreens with favourable N/P ratio promoting the decomposition of deciduous litter).

In early studies on species mixture effects on decomposition usually 50–50 mixture by mass were employed and in any trait-based predictions (Hoorens et al. 2010) all species got equal weight. Recently, studies related to carbon gain and loss processes in communities have started to weight species by their relative abundance in order to better approach vegetation composition in the field. The evergreen and deciduous species mixed in litter can affect two components of the trait composition for litter decomposition (De Bello et al. 2009): (i) the community-weighted mean of trait values (CWM), which is the average of the values of a trait of species present in litter mixture (Garnier et al. 2004; Ricotta and Moretti 2011) and (ii) functional diversity, which reflects the variation in values of a specific trait, or sets of various traits, of species within a mixture (Schleuter et al. 2010). Two particularly meaningful parameters to represent such trait variation may be the abundance-weighted functional (trait) evenness and the abundance-weighted functional (trait) dissimilarity, where the latter is indicated by Rao's quadratic entropy index (Botta-Dukát 2005; Lepš et al. 2006). For subtropical broad-leaf forest, we hypothesized that (1) overall decomposability of litter types, not including interactions, should be predicted by the CWM of specific leaf area (SLA) as a general predictor of decomposability of deciduous species (high SLA, high decomposability) to evergreen species (low SLA and decomposability); (2) the size of any positive non-additive effect of litter mixing on decomposition rate, and associated N release, should be predicted by the functional diversity in terms of litter N/P ratio. This functional diversity may predict increasing mixing effects with increasing differences in concentrations of key resources to decomposer organisms, and can be represented by two parameters. The first parameter is the abundance-weighted dissimilarity, as indicated by Rao's quadratic entropy index for N/P ratio between the litters in the mixture. We expect a positive linear relation of $Rao_{N/P}$ with the effect size of non-additivity in decomposition rate and nitrogen release. The second parameter is the CWM of litter N/P ratio. Here we expect that a hump-back shape, with the highest effect sizes for non-additivity in decomposition rate or nitrogen release at 50–50 deciduous-evergreen

mixtures, will represent the model for functional evenness in litter N/P ratio. We tested these hypotheses in a subtropical broad-leaved forest in eastern China. A unique feature of this experiment is that we used combinations of three deciduous species and/or three evergreen species, respectively, in each sample, thereby reducing chance effects of less representative deciduous or evergreen species. We carried out a one-year field decomposition experiment, with multiple harvests, with a range of five litter types ranging from three evergreens only to three deciduous broadleaf species only, with 30:70, 50:50 and 70:30% mixtures of these two extremes in between.

Materials and methods

Study site and forests

We established experiment in Tiantong National Forest Park (29°52′N, 121°39′E), located in the Ningbo coastal area, Zhejiang Province, in Eastern China. The climate of this region is subtropical monsoon. Mean annual temperature is 16.2 °C, with a January minimum of 4.2 °C and a July maximum of 28.1 °C. Mean annual precipitation is 1374.7 mm, more than 80% of it falls in the wet season (from April to September) and 20% in the dry season (from October to March) (Wang et al. 2007). The climax vegetation in this region is subtropical evergreen broad-leaved forest (EBLF). However, in the valley and footslope areas, evergreen-deciduous broad-leaved mixed forests are dominant due to favourable soil fertility and hydrology. In addition, deciduous tree species often invade into secondary EBLFs after natural and human disturbance, thus leading to a widespread distribution of mixed evergreen-deciduous broad-leaved forests currently. Species belonging to *Fagaceae*, *Theaceae*, *Ulmaceae* and *Hamamelidaceae* are dominant components of evergreen-deciduous broad-leaved mixed forests at the study site and six of these were selected for the litter decomposition experiment.

Experimental design and litter decomposition process

In summer 2014, we measured SLA of six species. Five branches were cut from different canopy positions for five randomly selected plants per species in the studied forest. Mostly, 20 undamaged mature leaves were

selected, stored in sealed plastic bags, and kept cool until brought back to the laboratory. Within 12 h, those samples were scanned using a leaf area meter (LI-3100C, Li-Cor, USA) to determine the mean leaf area (Pérez-Harguindeguy et al. 2013). Then the samples were dried at 75 °C in an oven for 48 h to determine leaf dry mass, which was then used to calculate SLA.

In order to collect leaf litters, three individual trees of each of the six species were randomly selected and 5~6 litter traps were installed under the canopy of each individual tree in the semi-intact EBLF. Freshly senesced, undecomposed leaves were collected from each litter trap every month in autumn 2014 (deciduous species) and every month in winter 2014 (evergreen species) from each individual tree of each species. The selected evergreens were *Cyclobalanopsis myrsinifolia* (Fagaceae*), Castanopsis fargesii* (Fagaceae) and *Schima superba* (Theaceae) while the three deciduous species were *Aphananthe aspera* (Ulmaceae), *Celtis sinensis* (Ulmaceae) and *Liquidambar formosana* (Hamamelidaceae).

The leaf litter collections were sorted and cleaned, then air-dried in the laboratory for 30 days to ensure that they reached stationary moisture content. Subsamples of each leaf litter species were dried at 75 °C for 48 h and used for initial chemical composition determination and measurement of the initial water content, needed to estimate the air-dry to oven-dry mass ratio. The other part of each litter species was used in the decomposition experiment.

In July 2014, we established three 10 m × 20 m replicate plots (with distance 10 m between plots) within a semi-intact EBLF, and each plot hosted five 1 m × 2 m subplots (15 subplots in total). Subplots had a 2 m buffer strip to minimize interactive effects between samples of different mixture treatments. The 15 subplots were chosen to have similar slope, altitude, soil type and substrate as far as possible. We removed the herbaceous and the litter layer from these plots.

We created 5 litter mixtures of approx. 27 g each with different proportions of evergreen to deciduous litters. In each litter mixture treatment, three individual litter species were evenly distributed within the evergreen and deciduous components, respectively. The percentages of evergreen mixtures versus deciduous mixtures were 100:0 (E100), 70:30 (E70D30), 50:50 (E50D50), 30:70 (E30D70) and 0:100% (E100) (see Table S1 for details). Each litter treatment had three replicates, each of which was placed in a separate subplot, with nine

harvest times for each litter combination (the total number of litter bag samples was $5 \times 3 \times 9 = 135$).

Each mixed leaf litter sample was sealed into a 20 cm × 25 cm nylon mesh cloth (pore size 1 mm × 1 mm), after its actual air-dry mass (27 g ± 0.1 g) had been weighed. The litterbags of each of the five treatments were placed on the forest floor in their respective subplots within each of the three replicate plots, and each treatment replicate had nine leaf litter bags in each subplot, i.e. one for each harvest. Each plot was covered with gauze (placed above about 1 m high) in order to prevent new litter to fall in. On Nov. 11, 2014, all 135 litter mixture bags were pinned to the soil surface in the 15 subplots. The litter bags were collected from each plot in, respectively, weeks 4 (Dec.), 8 (Jan), 16 (Mar), 24 (May), 28 (June), 32 (July), 36 (Aug), 44 (Oct), and 52 (Nov. 11, 2015) of the incubation. Because we assumed decomposition to be faster initially (leaching phase) and during the summer (warm conditions), we gathered monthly in the first two months and the three summer months (June, July, August), for the other periods every two months.

After retrieving the litter bags, we removed extraneous materials from the decomposed leaf litter samples, dried them in an oven 75 °C for 48 h, and then weighed. Mass remaining at each harvest was expressed as % of initial oven-dry mass. Then, litter samples were pulverized through a fine sieve for further chemical analyses.

Litter chemical analyses

Both initial leaves and decomposed litters after each harvests were grounded by using a laboratory mill and then passed through a 0.15 mm sieve. Total carbon (C) content was measured by combusting 10 mg subsamples on an Elementar (vario TOC, Elementar, Germany). Thereafter, 0.2 g sub-samples were digested using concentrated H_2SO_4 to determine N and P concentrations on an infrared spectrophotometer (Smartchem 200, Alliance, France).

Calculations and statistical analyses

We used the leaf litter decay coefficient k and nutrient release % to quantify key litter decomposition parameters. Litter decomposition rate k-value was calculated by fitting the exponential decay function $y = a \times e^{-kt}$ (Olson 1963), where y is the % dry mass remaining, a is the

fitting parameter, k is the decay coefficient, and t is decomposition time (week). Litter C, N, P release% were calculated according to Eq. 1:

$$100 \times \left[(M_i \times CNP_i) - (M_f \times CNP_f) \right] / (M_i \times CNP_i) \tag{1}$$

where M_i and M_f are the initial and final litter dry mass, and CNP_i and CNP_f are the initial and final C, N, P concentrations.

In order to test how functional composition and diversity affect litter decomposition, Rao's Q and CWM (see Introduction) were calculated for different litter combinations (Eqs. 2 and 3, respectively). We considered that Rao's Q quadratic entropy is a suitable index to quantify the trait dissimilarity among species.

$$Rao_j = \sum_{i=1}^{n} \sum_{k=1}^{n} d_{ik} p_i p_k \tag{2}$$

where n stands for species number within mixtures, d_{ik} is the dissimilarity coefficient based one Euclidean distance between two species i and k in the multivariate trait space of litter mixture j (García-Palacios et al. 2017), and p_i and p_k are the relative abundances of species i and k in the litter mixture j, respectively.

$$CWM_j = \sum_{i=1}^{n} p_{ij} \times t_{ij} \tag{3}$$

where p_{ij} is the relative abundance of species i in the litter mixture j, and t_{ij} is the mean trait value of species i in the litter mixture j.

We used one-way analysis of variance (ANOVA) to test the effects of litter functional composition and diversity (fixed factor) on decomposition rate (k-value), C, N, P release %. We examined the assumptions of normality and homogeneity by performing Shapiro-Wilk and Levene's tests, respectively. We also tested for homogeneity of variance in the mixture effect size of the five treatments. If the distributions did not deviate from homogeneity of variance, we used the LSD to do paired comparisons, if not, we applied Dunnett's tests.

To test the non-additive effects of species combinations on litter decomposition rate k, and on the C, N, and P release %, we first drew our null hypothesis using 100% evergreen and 100% deciduous treatments with CWMs, calculating a linear relationship between them as the expectation without mixture effect; then we calculated curvilinear models with all treatments and CWMs as observations. The mixing effect size was calculated by the absolute difference between observation and expectation, since the underlying response variables were already percentages themselves. Then we tested the differences in mixture effect size among the five treatments.
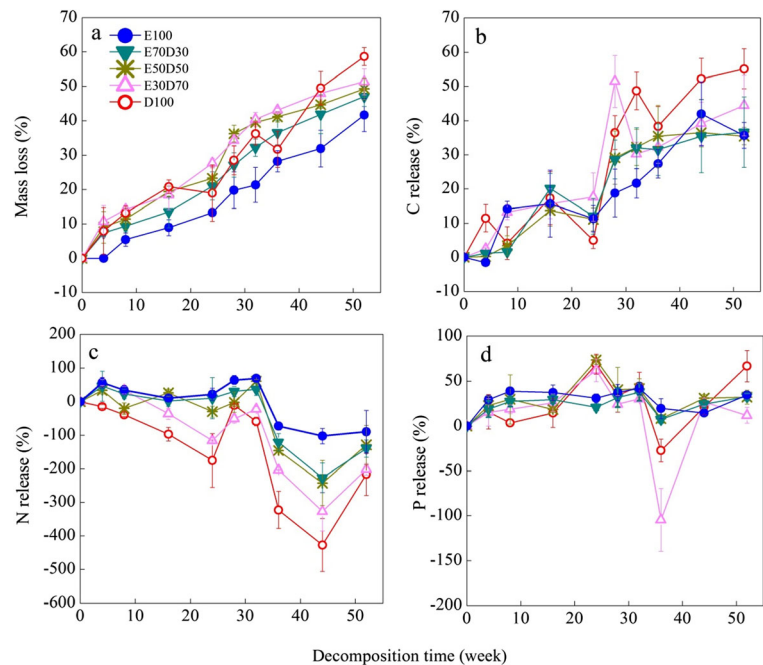
In order to quantify the relationship between the mixing effect size and Rao functional trait dissimilarity values, we used linear and/or non-linear regressions to select the best model between CWMs or Raos with decomposition rate (k-value), C, N, and P release %, respectively. The best model was selected according to the lowest Akaike information criteria (AIC). All statistical analyses were conducted using the R software, v.3.4.1 (R Core Team 2014), with which the lme4 package was used for non-linear regressions, and model selection was performed by using the 'dredge' function of the MUMIN package.

Results

Mass loss and nutrient release or immobilization in mixtures varying in percentages of evergreen versus deciduous litter

On average, the N/P ratio of leaf litters was significantly higher, but SLA was lower in evergreen than deciduous species (see Table S2 for details). The general pattern of leaf litter mass loss between the five treatments varied widely and showed a consistent upward trend during the 52-week decomposition period, although the pattern fluctuated strongly in the 100% deciduous mixture (D100) (Fig. 1a). Total organic C release % appeared to broadly follow the gradual increasing pattern for mass loss %, also in terms of actual values, but the variability among replicates and fluctuations through time obscured any clear difference among treatments (Fig. 1b). Evergreen 100% (E100) had the lowest mass loss % with the highest N release % (or, later, least N immobilization) compared to other treatments throughout experimental time. Interestingly, while D100 did not have the highest mass loss % through time (although it did by the end, after 52 weeks), it had the most negative N release % (Fig. 1c), indicating the strongest N immobilization, among all treatments. In contrast, there was no obvious consistent sequence of C release % (Fig. 1b)

and P release % (Fig. 1d) among treatments in the two litter extremes (E100 and D100). Mass loss % was higher in mixtures with lower proportion of evergreens (other than D100) throughout the experiment, i.e. the mass loss % order was: E100 < E70D30 < E50D50 < E30D70. The pattern for N release % was opposite, N release % was higher in mixtures with higher proportion of evergreens: E100 > E70D30 > E50D50 > E30D70 > D100. This meant that the order for mass loss among treatments broadly matched that for N immobilization. The E100 and E70D30 treatments had a considerable initial P release % but no net release or immobilization later on, while the other treatments fluctuated greatly between P release % and immobilization during the decomposition process (Fig. 1d).

The patterns of cumulative N immobilization and release depended on the initial N concentration of litter mixtures, as N immobilization occurred in the mixtures with lower initial N concentration, i.e. D100 and E30D70 (Table S3). In these treatments N was immobilized the most strongly as the mass loss increased (Fig. 1a, c), but they showed N release % in weeks 24~32 (summer season) and by week 52 (winter). These shifts between N immobilization and release were more moderate in the other treatments.

First-order and non-additive effects on litter mixture decomposition

While CWM_{SLA} was a good first-order predictor of decomposition rate and N release %, in support of our first hypothesis, this relationship was not linear. Instead, there were tight hump-backed relationships of k-values (positive, $R^2 = 0.73$, $P < 0.001$) and N release % (negative, $R^2 = 0.89$, $P < 0.001$) with CWM_{SLA} (Fig. 2). In other words, there was an obvious trend that the observed values in deciduous-evergreen mixtures were bigger than predicted values (based on absence of mixture effect; i.e. linear relationship) in the relationship between k-value and N release % with CWM_{SLA}, indicating that litter mixing caused positive mixing effects on k-value and N release % (Fig. 2). In contrast, there was no obvious relationship of C release % (not shown) or P release % with CWM_{SLA} ($P > 0.05$, Fig. 2).

Related to our second hypothesis, functional evenness turned out to be a much better predictor of non-additive effects on decomposition processes than functional dissimilarity. Indeed, hump-backed curves could also explain the relationships between the relative mixing effect size of k values or N release % and $CWM_{N/P}$ tightly ($R^2 = 0.72$ and 0.95 respectively; see Fig. 3). In contrast the mixing effect size of k or N release % was only weakly positively correlated with
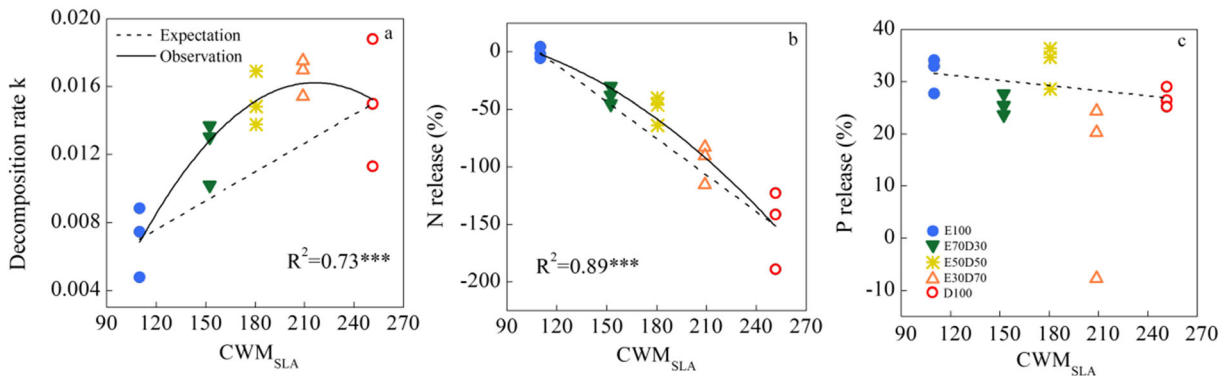
Fig. 2 The relationship between CWM_{SLA} and decomposition rate k-value, N release % and P release % of different treatments.*$P < 0.05$; ** $P < 0.01$ ** $P < 0.001$. Color version of this figure is available online

Rao's dissimilarity for N/P ($R^2 = 0.38$ and 0.27 respectively; Fig. 3) while it was not significantly correlated with Rao for SLA (data not shown). Between the different mixture treatments, the positive relationships between k value and N release % respectively with $Rao_{N/P}$ were strongly driven by the high values for E50D50, i.e. for the treatment with particularly high functional dissimilarity. In contrast, there was no obvious relationship between the mixing effect on P release % with $CWM_{N/P}$ ($P > 0.05$) or $Rao_{N/P}$ ($P > 0.05$).

Discussion

This study provides empirical support for our two hypotheses concerning the predictive power of leaf litter traits for variation in decomposition rates. Indeed, in support of hypothesis 1, the community-weighted mean (CWM) of specific leaf area could broadly explain variation in decomposition rates in a range of litter mixtures varying in the proportions of evergreen and deciduous species. We will not discuss this finding in-depth, as it broadly corresponds to previous, worldwide findings (Gallardo and Merino 1993; Cornelissen et al. 1999; Cornwell et al. 2008, using 1 divided by leaf mass per area for SLA; although (Fortunel et al. 2009) found this relationship within some but not all of their European sites. Instead, we will focus the discussion on a more contentious issue, i.e. species interactions in litter mixtures (i.e. hypothesis 2). We hypothesized that the functional diversity in litter N/P ratio would predict the effect size of non-additivity in decomposition rate and N release %, and proposed two alternative models to represent functional diversity. While there were strong

positive non-additive effects of evergreen-deciduous litter mixing on decomposition rates (k) and associated nutrient (especially N) release %, these non-additive effects showed significant but relatively weak relationships with the first functional diversity parameter, i.e. litter functional dissimilarity in terms of the Rao index for litter N/P ratio. In contrast, there was a very tight fit with our second proposed model: we found a rather tight hump-backed relationships of k and N release % with the CWM of litter N/P ratio across a range of mixtures from predominantly deciduous to predominantly evergreen species. As the hump of these curves represents the most diverse combination(s) in terms of deciduous and evergreen proportions, this finding does provide convincing support for functional diversity in terms of N/P ratio as a predictor of positive non-additivity. The fact that the mass ratios of deciduous-evergreen mixtures were transferred to abundance-weighted trait means makes that our findings have a closer connection to the actual mechanisms of mixture effects on decomposition. Below we will discuss these findings, and its limitations and promises, in the global ecological context of the previous literature.

Nutrient content and N/P ratio of deciduous species versus evergreens

Evergreen and deciduous species have different initial nutrient status. Globally, species with longer leaf life span, as in most evergreens, tend to have lower fresh leaf N concentrations than species with shorter leaf lifespan, as in most deciduous plants (Reich 2014). During leaf senescence, evergreen species are often assumed to have higher nutrient resorption efficiency
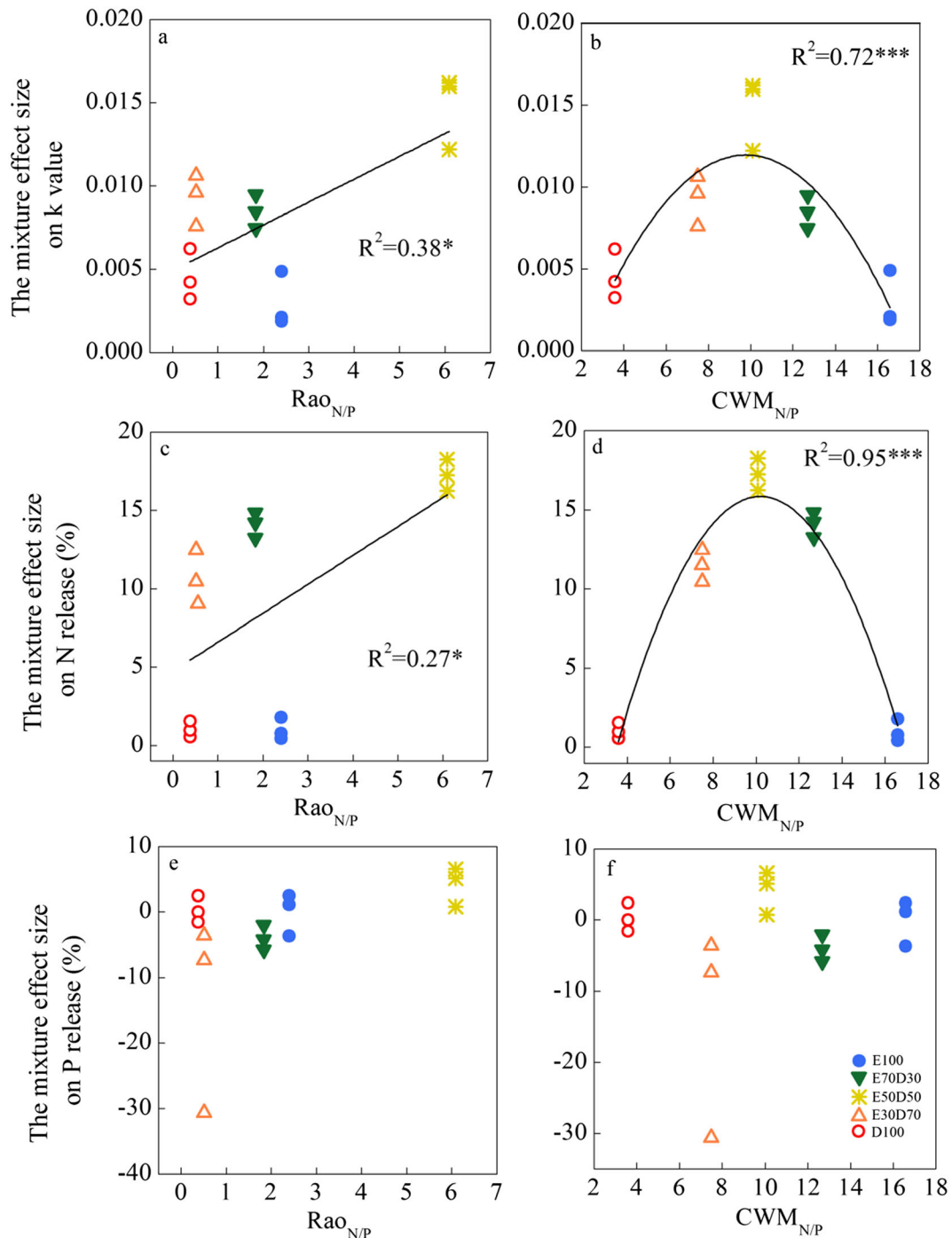
Fig. 3 The relationships between mixture effect size of decomposition rate k-value and N, P release% in different treatments respectively, with $Rao_{N/P}$ and $CWM_{N/P}$. *$P < 0.05$; ** $P < 0.01$ ** $P < 0.001$. Color version of this figure is available online

than deciduous species so as to reduce nutrient loss (Aerts and Chapin 1999), although in actual fact N resorption efficiency tends to be slightly higher in higher-latitude deciduous than evergreen species

(Aerts 1996). In contrast, in our study region (EBLFs in subtropical China), some of the predominant deciduous species do not have higher leaf N content than evergreens. Also, they showed a slightly greater N

resorption efficiency than evergreens (e.g. $NRE_{decidous}$ = 38.72, $NRE_{evergreen}$ = 33.91 in Huang et al. 2007). As a consequence, litter N concentrations of deciduous species are lower than those of evergreens in this region. This difference is even amplified in our study species. In general, *Liquidambar formosana*, *Celtis sinensis*, and *Aphananthe aspera* predominate degraded habitats and valley areas, characterized by low soil N availability (Yan et al. 2006), which puts a premium on high N resorption efficiency (Yan et al. 2010). These factors may explain why litter N concentrations of deciduous species are much lower than those of evergreens in this study (Table S2). Combining this with the litter P concentrations, which were generally similar or even higher in deciduous compared to evergreen species, litter N/P ratios were much lower in deciduous than in evergreen species in this study (Table S2). As we will discuss below, these initial differences in litter N/P ratios are key to understanding non-additive effects on decomposition of deciduous-evergreen litter mixtures.

Effect of diversity in litter N/P ratio on non-additivity in litter decomposition

Our results demonstrated that mixed litter decomposition was subjected to obvious non-additive effects. This finding is consistent with several previous studies that have shown a non-additive effect of litter mixtures on decomposition, both in aquatic ecosystems (terrestrial tree leaf litters were placed in streams, Kominoski et al. 2007) and terrestrial ecosystems (see Introduction). Here we advance the understanding of such non-additivity for mixtures of deciduous and evergreen litters, by applying a range of relative abundances of these two litter types in mixtures. In general, due to the complexity of woody species composition in natural forests, the relative proportions of deciduous and evergreen species vary extensively in subtropical SE Asia, as well as in many forested ecosystems worldwide. Our experimental design aimed to capture this broad variation in natural forests. Especially, our study points to a likely mechanism by which such non-additive effect is dependent on the functional diversity and composition in the evergreen-deciduous litter mixtures. As hypothesized, the mixing effect sizes of k value and N release % were positively related to the $Rao_{N/P}$ as they increased steeply with $Rao_{N/P}$ from any of the four other mixture treatments to E50D50. However, functional dissimilarity in terms of $Rao_{N/P}$ was not the best predictor of the effect size of non-additivity of litter decomposition. This is possibly because $Rao_{N/P}$ was

sensitive to variance in k values or N release %. Indeed the variance in k values or N release % within the three species of evergreens and within the three deciduous species, respectively, was much greater than the variance within each of the mixture treatments (see Fig. 2). Especially, $Rao_{N/P}$ of the E100 treatment, i.e. three evergreen species alone, was higher than that in the D30E70 treatment, presumably because the high N/P ratios of the evergreens (compared to those of deciduous species) led to a high absolute variance and thus a high $Rao_{N/P}$. In contrast, the tight hump-backed relations of the effect size of k or N release % with $CWM_{N/P}$ were less sensitive to the variance in N/P among species within leaf habits. The community-weighted mean puts more emphasis on the evenness of trait values. Thus, the effect sizes of positive non-additivity of litter decomposition and N release % were best described by functional evenness first increasing and then decreasing according to a hump-backed relationship with $CWM_{N/P}$.

The ecological mechanism underlying the positive relationship between the size of mixing effect and functional diversity, especially functional evenness, might be attributable to niche complementarity among mixed species (Díaz et al. 2007; Schimel and Hattenschwiler 2007; Handa et al. 2014). In our study, niche complementarity played a likely role in promoting the non-additive effects of litter mixture on decomposition and this role was most likely associated with the nutrient transfer process in evergreen-deciduous mixed leaf litters. Relative to evergreens, lower initial N content and N/P in deciduous leaf litters (Table S2) suggest that litter decomposition of deciduous species was limited more by N. Indeed, when two types of leaf litters were combined, the N released from evergreens offset the N limitation in deciduous litters somewhat. For example, through one year, D100 and E30 D70 kept a consistent pattern of N immobilization across seasons, while E100 and E70D30 showed some N release in the first 7 months of field incubation (Fig. 1). The relative shift from N immobilization to N release corresponded nicely with the proportion of evergreens in the litter. This result provides clear albeit indirect evidence for litter N-transfer from evergreen to deciduous litters. It also explains why P release, or the effect size of non-additivity on P release, was not predicted by the proportions of evergreen versus deciduous litter species. At the same time, the generally fast decomposition of deciduous litters (Figs. 1 and 2) may have created an active microbial community and some summer P release (Fig. 1d), stimulating the decomposition of evergreens in mixtures. The litter nutrient (especially N) transfer and microbial

facilitation between evergreens and deciduous would theoretically have the strongest positive effect on litter decomposition when the two types of leaf litter are mixed evenly. This was indeed the case as the litter mixtures with more even distribution of N/P in functional space facilitated decomposition and N release % more (or reduced N immobilization more), thus showing the greatest size of non-additive effects of litter mixture. Thus, the litter mixtures with the best stoichiometric balance caused the greatest non-additive effect size on mass loss and N release %. We acknowledge that the microbial mechanisms behind the litter nutrient transfer processes between evergreen and deciduous species should be investigated in depth in future, for instance through isotopic labeling.

Conclusions

By using five leaf litter mixtures with contrasting N/P and SLA from three evergreen and three deciduous species in the subtropical area of China, this study has tested how functional diversity and composition affect litter decomposition as related to two key plant strategies. We observed that functional composition in terms of community-weighted SLA is a main predictor of increasing litter decomposition rate; and of increasing N immobilization because of low N/P ratio of deciduous tree species in subtropical China. There are remarkable positive non-additive effects of litter mixture on litter decomposition. The hump-backed relationships of the mixing effect size of decomposition rate and N release $CWM_{N/P}$ suggest that litter mixtures with the highest functional evenness can cause the greatest size of non-additive effects of litter mixture, due to a combination of N transfer from N-rich evergreen litters to N-poor deciduous litters and facilitation of evergreen litter decomposition by high microbial activity deciduous in fast-decomposing deciduous litter. This study provides strong evidence that differences in N/P stoichiometry between evergreen-deciduous leaf litter mixtures play a strong role in influencing litter decomposition rate nutrient release, which are among the key ecosystem processes in terrestrial ecosystems. Moreover, our study highlights that the community weighted trait mean not only has great relevance and predictive power for carbon fixation and growth related processes (Grime 1998; Garnier et al. 2004), but also for carbon and nutrient release processes in ecosystems. Overall, the results in this study may benefit our understanding of how functionally

different species, especially in terms of the key plant strategies, interactively control the decomposition of litter mixtures.

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