Synergistic effects: a common theme in mixed-species litter decomposition

Jun Liu[1,2], Xiaoyu Liu[2], Qingni Song[3,4], Zacchaeus G. Compson[5], Carri J. LeRoy[6], Fenggang Luan[1], Hui Wang[7], Yalin Hu[2] and Qingpei Yang[3,4]

[1]College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China; [2]College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, China; [3]Jiangxi Provincial Key Laboratory for Bamboo Germplasm Resources and Utilization, Jiangxi Agricultural University, Nanchang 330045, China; [4]2011 Collaborative Innovation Center of Jiangxi Typical Trees Cultivation and Utilization, Jiangxi Agricultural University, Nanchang 330045, China; [5]Centre for Environmental Genomics Applications, St John's, NL A1A 0R6, Canada; [6]Environmental Studies Program, The Evergreen State College, Olympia, WA 98505, USA; [7]College of Resources and Environment, Shandong Agricultural University, Taian 271018, China

Authors for correspondence:
Yalin Hu
Tel: +86 17720797616
Email: huyl@iae.ac.cn

Qingpei Yang
Tel: +86 13479161643
Email: qingpeiyang@126.com

Received: *25 October 2019*
Accepted: *10 March 2020*

New Phytologist (2020) **227:** 757–765
doi: 10.1111/nph.16556

Key words: antagonistic effects, litter decomposition, litter mixture, litter quality, meta-analysis, nonadditive effects, synergistic effects.

Summary

- Litter decomposition plays a key role in nutrient cycling across ecosystems, yet to date, we lack a comprehensive understanding of the nonadditive decomposition effects in leaf litter mixing experiments.
- To fill that gap, we compiled 69 individual studies with the aim to perform two meta-analyses on nonadditive effects.
- We show that a significant synergistic effect (faster decomposition in mixtures than expected) occurs at a global scale, with an average increase of 3–5% in litter mixtures. In particular, low-quality litter in mixtures shows a significant synergistic effect, while additive effects are observed for high-quality species. Additionally, synergistic effects turn into antagonistic effects when soil fauna are absent or litter is in very late stages of decomposition (near-humus). In contrast to temperate and tropical areas, studies in boreal regions show significant antagonistic effects.
- Our two meta-analyses provide a systematic evaluation of nonadditive effects in mixed litter decomposition studies and show that litter quality alters the effects of litter mixing. Our results indicate that nutrient transfer, soil fauna and inhibitory secondary compounds can influence mixing effects. We also highlight that synergistic and antagonistic effects occur concurrently, and the final litter mixing effect results from the interplay between them.

Introduction

Litter decomposition is a central component of ecosystem biogeochemical cycles. The rate of decomposition controls nutrient cycling and energy flow, which regulates atmospheric carbon emissions, soil organic matter composition and nutrient availability (Schneider *et al.*, 2012). Thus, litter decomposition influences ecosystem primary productivity (Bradford *et al.*, 2016). Over the past few decades, numerous litter decomposition studies have focused on single litter decay (Gartner & Cardon, 2004), providing an extensive exploration of the influence of various factors on decomposition rates. However, in many ecosystems, litter is generally a mix of multiple species. Previous studies suggest that litter species interact with each other during their decomposition (Ball *et al.*, 2008; Gessner *et al.*, 2010), implying that the decomposition rates of litter mixtures are different from those of single-species litters. Discerning how litter mixtures influence decomposition is essential to understanding carbon and nutrient cycles.

Litter species with different chemical compositions show distinct quality and decomposability, namely species-specific decomposition (Gartner & Cardon, 2004; Cornwell *et al.*, 2008). When these litter species mix together, their decomposition rates generally do not equal the arithmetic mean value of each species in isolation (i.e. the expected decay rate; Supporting information Fig. S1) (Gartner & Cardon, 2004; Steinwandter *et al.*, 2019). Instead, two alternative options are possible: a synergistic effect (faster decomposition in mixture than expected) or an antagonistic effect (slower decomposition in mixture than expected). Collectively these are referred to as nonadditive effects (Fig. S1). In general, the chemical characteristics of litter, decomposer activity and other environmental factors often constrain decomposition rates (Berg, 2014). In litter mixtures, the underlying mechanisms of synergistic effects are thought to be influenced by three main factors: nutrient transfer (e.g. nitrogen, phosphorus) from high-quality litter species to low-quality litter, the complementarity effects of soil fauna and decomposers, and the improvement of microclimatic conditions during decomposition (Madritch & Cardinale, 2007; Schimel & Hattenschwiler, 2007; Tiunov, 2009). Conversely, antagonistic effects are often induced by the enhancement of microbial nutrient immobilization for litters with poor nutrients or inhibitory secondary compounds released from low-quality litter species (Hättenschwiler *et al.*, 2005; Montané et al., 2013).

Although experiments concerning litter mixture effects on decomposition rates have been conducted in numerous individual studies, conflicting results have hampered any possibility to draw general conclusions (Li *et al.*, 2016). Conflicting results may stem from differences in experimental design (Barbe *et al.*, 2018; Leroy *et al.*, 2018; Zhao *et al.*, 2019). Thus far, studies on mixed litter decomposition have been performed in different ecosystems and across different climate regions, with decomposition durations varying from several weeks to several years. Moreover, various mesh sizes are used in litterbags and microcosms, the two methods that are most commonly employed in litter decomposition studies, further hampering our ability to compare results. As such, for the past several years, the scientific community has been requesting a meta-analysis to determine a general global pattern of mixed litter effects (Gartner & Cardon, 2004; Gessner *et al.*, 2010). While studies throughout the literature have summarized and analyzed nonadditive effects in mixed litter decomposition studies (Gartner & Cardon, 2004; Li *et al.*, 2016), many unanswered questions remain. To better understand how litter mixing influences decomposition rates, we employed meta-analytic methods to identify the central tendency of litter mixture effects on litter decay rates built upon analyses completed by Gartner & Cardon (2004).

We approached the meta-analyses with four main hypotheses: (1) if nonadditive litter mixing effects were observed, we anticipated that the effects would vary across different climate regions and ecosystems, because environmental factors strongly affect litter decay processes (Zhang *et al.*, 2008); (2) we expected that synergistic effects would be reduced for small mesh sizes and long study durations, as both of these factors could limit invertebrate decomposers, which have been shown to increase synergistic effects in litter mixtures (Jabiol & Chauvet, 2015); (3) we hypothesized that lower evenness and higher richness of litter species would be conducive to synergistic effects due to resource complementarity (Gessner *et al.*, 2010; Otsing *et al.*, 2018); and (4) we hypothesized that different qualities of litter (leaf types, leaf families) would show different responses in litter mixtures, because higher quality litters could share nutrients with lower quality litters to promote decomposability while lower quality litters might transmit specific secondary compounds (tannins or phenolics) to impede high-quality litter decay (Gao *et al.*, 2016). We compiled data from 69 individual studies to perform two global meta-analyses to test these hypotheses (Notes S1).

Materials and Methods

Data compilation

Publications that have reported data on litter mixture decomposition rates were selected from the Web of Science, Google Scholar and the China National Knowledge Infrastructure. Boolean keyword searches consisted of term combinations, such as '(litter or debris or residue) AND (mix* or diversity or nonadditive effect or additive effect) AND (decompos* or decay* or degrad*)'. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram provided the procedure used for the selection of studies for the meta-analyses (Fig. S2).

Each selected publication had to satisfy the following three criteria: it had to report at least one of our selected variables (expected decay rate (R_{exp}) vs observed decay rate (R_{obs}), or species-specific decay rate in mixture R_{mix} vs singular (R_{sin})), with the decay rate expressed as mass loss or mass remaining (%); it had to provide the means and sample sizes (n) of the variables selected for the meta-analyses or the possibility to calculate n; and the measurements of selected variables had to be performed at the same temporal and spatial scale. For all chosen studies, except for litter mixture treatments, other experimental treatments (such as nutrient addition, warming, water controlling) were excluded. If the data were presented in figures, GetData Graph Digitizer (v.2.24) was used to extract numerical values. In addition, when the selected variables used to estimate the nonadditive effect were not provided in the papers, we emailed the corresponding authors to query the original data of k values (yr^{-1}) or relative mixture effect ((observed mass loss − expected mass loss)/expected mass loss × 100).

We partitioned the dataset to perform two meta-analyses (Fig. 1). Meta-analysis 1 compared decay rates of litter mixtures between expected decay rates (R_{exp}: additive rates determined by averaging all litter decomposition rates for singular species) and observed decay rates (R_{obs}) for mixed litter treatments. Meta-analysis 2 compared species-specific decay rates between single species treatments (R_{sin}) and mixture treatments (R_{mix}). Both R_{obs} and R_{mix} represent actual results from litter mixture studies, but R_{obs} values are rates for the whole mixture while R_{mix} values are rates for each species in each mixture separated and compared to single-species values. The additive effect and nonadditive effect occur when 'R_{exp} vs R_{obs}' or 'R_{sin} vs R_{mix}' are equal and not equal, respectively. Hypotheses 1–3 were tested by both meta-analyses 1 and 2, while hypothesis 4 was only tested by meta-analysis 2. In total, 873 paired observations in meta-analysis 1 were obtained from 53 selected papers and 270 paired observations in meta-analysis 2 were obtained from 16 papers (Table S1). These studies were primarily conducted in East Asia, Western Europe and North America (Fig. S3).

For each study, we also noted the factors relevant to the mixing effects, including site location, ecosystem type, litter species (and its nutrient contents if provided), decay duration (days), mesh size and other background information (mean annual temperature (MAT), mean annual precipitation (MAP), etc.).

Statistical analysis

The natural log-transformed response ratio (R), defined as the effect size (Hedges *et al.*, 1999), was used as an index to measure the effect size of the litter mixture on decomposition rate:

$$\log_e R = \log_e\left(\overline{\mathrm{Xt}}/\overline{\mathrm{Xc}}\right) = \log_e\left(\overline{\mathrm{Xt}}\right) - \log_e\left(\overline{\mathrm{Xc}}\right) \qquad \text{Eqn 1}$$

where $\overline{\mathrm{Xt}}$ and $\overline{\mathrm{Xc}}$ represent R_{obs} and R_{exp}, respectively, or R_{mix} and R_{sin}, respectively.

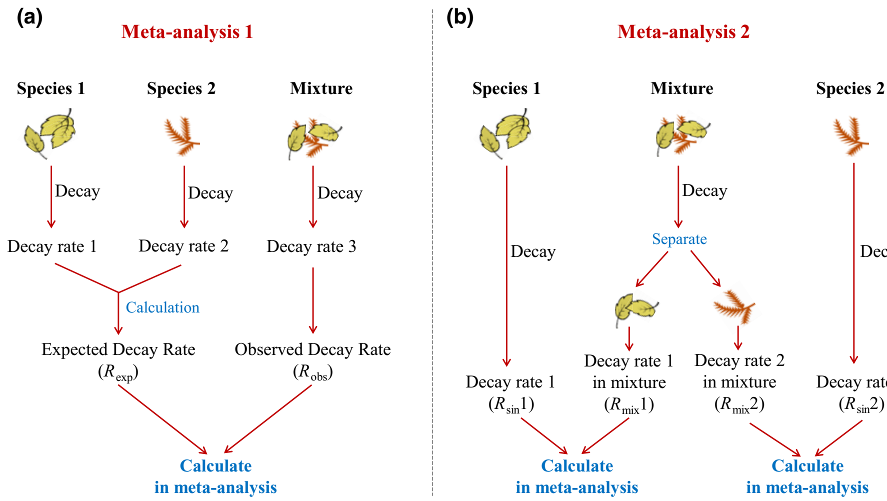

Fig. 1 The two meta-analyses in this study. (a) Meta-analysis 1, a comparison of litter mixture decay rates between expected rates (R_{exp}) and observed rates (R_{obs}). $R_{exp} = w_1R_1 + w_2R_2 + \ldots + w_nR_n$, where w_n is the weight of species n in the mixture and R_n is the decomposition rate of species n. (b) Meta-analysis 2, a comparison of species-specific decay rates between single species (R_{sin}) and species mixture (R_{mix}) decomposition rates. The additive effect and nonadditive effect are when 'R_{exp} vs R_{obs}' or 'R_{sin} vs R_{mix}' are equal and not equal, respectively.

The variance of $\log_e R(v)$ was calculated using:

$$v = \frac{S_t^2}{n_t \bar{X}_t^2} + \frac{S_c^2}{n_c \bar{X}_c^2} \quad \text{Eqn 2}$$

where S_t and S_c are the standard deviations (SDs) for R_{obs} and R_{exp}, or for R_{mix} and R_{sin}, respectively; n_t and n_c are the sample sizes for R_{obs} and R_{exp}, or for R_{mix} and R_{sin}, respectively. If both the SD and standard error (SE) were lacking, we estimated the missing SD by multiplying the average coefficient of variation (CV) from each data set by the reported mean value (Wiebe *et al.*, 2006).

A nonparametric weighting function was used to weight each individual study, and the mean effect size ($\overline{\log_e R}$) of all observations was estimated according to:

$$\overline{\log_e R} = \frac{\sum_i \log_e R_i}{\sum_i w_i} \quad \text{Eqn 3}$$

where w is the weighting factor used to calculate the inverse of the pooled variance ($1/v$), and $\log_e R_i$ and w_i are the $\log_e R$ and w of the ith observation, respectively.

To determine whether there was a significant difference from additivity under litter mixture treatments (Rosenberg *et al.*, 2000), we employed a fixed-effect model to calculate 95% confidence intervals (CIs) of the weighted effect size, using the METAWIN 2.1 software. The effect was only considered significant if the 95% CI values did not overlap 0. Furthermore, to clearly express nonadditive effects, the mean effect size was converted back to percentage change, using:

$$\text{The percent change} = \left(e^{\overline{\log_e R_i}} - 1\right) \times 100\% \quad \text{Eqn 4}$$

We grouped the data according to climate zones (tropical, temperate, boreal), ecosystem types (forest, shrubland, grassland, aquatic, peatland), mesh sizes (small (diameter < 1 mm), middle (1 mm ≤ diameter < 5 mm) and large (diameter ≥ 5 mm)), and decay periods (<180 d, 180–360 d, 360–720 d, > 720 d) (Knorr *et al.*, 2005). Particularly, in meta-analysis 1, we classified each litter mixture as even or uneven mixing according to the initial litter species ratio. In meta-analysis 2, litters with 10, 10–20 and > 20% initial lignin content were divided into the high, medium and low litter-quality categories, respectively (Knorr *et al.*, 2005). We also grouped trees and shrubs based on different functional types (broadleaf/needle, and evergreen/deciduous).

A continuous randomized-effect model was used to assess the potential linearity or nonlinearity between $\log_e R$ and climate factors (MAP and MAT) or forcing factors (decay period, and initial nutrient content). The total $\log_e R'$ heterogeneity among the selected studies (Q_T) was partitioned into different groups based on cumulative effect sizes (Q_M) and the residual error (Q_E) (Rosenberg *et al.*, 2000).

Results

The mean effect size calculated across all studies was significantly positive both in R_{exp} vs R_{obs} and in R_{sin} vs R_{mix}, with an average increase of + 3% and + 5%, respectively (Fig. 2a). When considering different climate zones, mixed litter decomposition showed a significant positive response in temperate zones and a significant negative response in boreal zones in both meta-analyses (Fig. 2b). Unlike temperate and boreal zones, in tropical zones R_{exp} was significantly higher than R_{obs}, and R_{sin} and R_{mix} differed little. The continuous randomized-effect model suggested that MAT had a significant positive correlation with the mean effect size in meta-analysis 1 (Table 1). With respect to ecosystem type, the mean effect sizes of decomposing litter mixtures were significantly positive (i.e. $R_{exp} > R_{obs}$) for all five ecosystems except shrublands (Fig. 2c).

Experimental conditions also influenced the decomposition of litter mixtures. For example, the mesh size of the litter packs influenced the decomposition rate of litter mixtures (Fig. 3). When mesh size was divided into three groups (small, middle and large), a marked antagonistic effect was found in studies using small mesh sizes (i.e. $R_{exp} < R_{obs}$). By contrast, decomposition rates of studies using medium and large mesh sizes showed strong synergistic responses (Fig. 3a). For meta-analysis 2 (R_{sin} vs

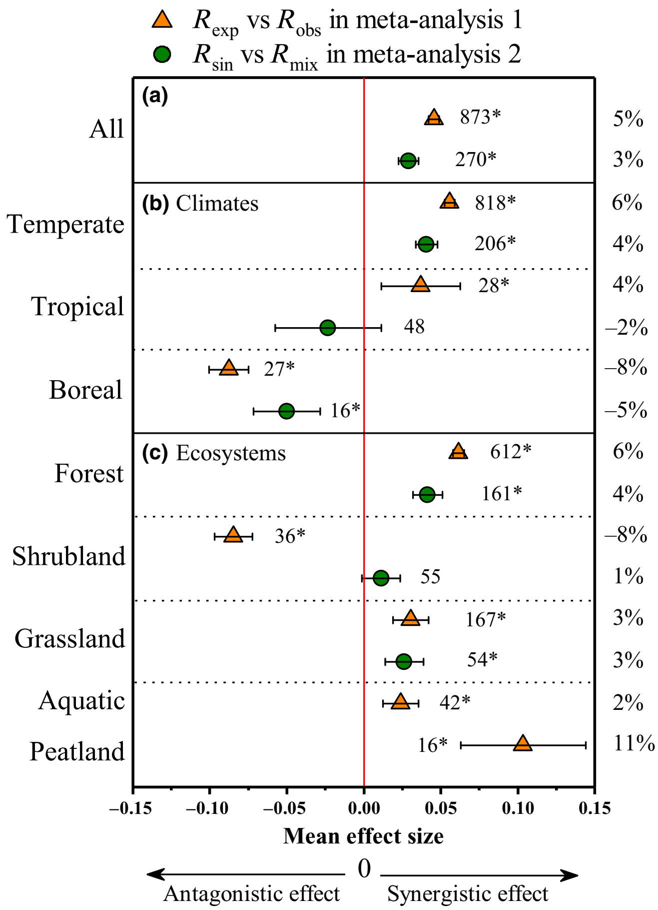

Fig. 2 Comparison of mixing litter decay rates between expected values (R_{exp}) and observed values (R_{obs}) (meta-analysis 1, orange triangles), and comparison of species-specific decay rates between single (R_{sin}) and in mixture (R_{mix}) decomposition (meta-analysis 2, green circles) (a) across all studies, (b) among different climates and (c) among different ecosystems. If the mean effect size = 0, then there is an additive effect; if the mean effect size > 0, then there is a synergistic effect; and if the mean effect size < 0, then there is an antagonistic effect. If the effect size 95% CIs (error bars) do not include zero, the nonadditive effect was considered to be significant (*). The sample size for each variable is shown next to the point. The data on the right-hand *y*-axis represent the mean percentage difference for each variable (%).

R_{mix}), the small and large mesh sizes showed an additive effect, whereas the middle mesh size showed a + 5% increase in decomposition rate (Fig. 3a). When the decay period was partitioned into four levels, the results showed synergistic effects of litter mixtures for short- (<180 d) and medium-duration (180–360 and 360–720 d) studies; conversely, a significant antagonistic effect was observed for long-duration (>720 d) studies (R_{exp} vs R_{obs}: −4%; Fig. 3b).

Additionally, the characteristics of the leaf litter itself influenced the decomposition of litter mixtures. In meta-analysis 1 (R_{exp} vs R_{obs}), both even and uneven litter mixtures showed synergistic effects on decomposition rate (Fig. 3c). In meta-analysis 2 (R_{sin} vs R_{mix}), when litter quality was divided into three levels based on lignin content (low, medium and high), the decomposition rate of low-quality litter exhibited a greater positive change

Table 1 Relationships between the effect size of litter mixing on decay rate and mean air temperature (MAT), mean annual precipitation (MAP), experiment duration, species richness and litter initial nutrient contents.

	Q_T	Q_M	Q_E	Slope	*P*-value	Sample size
In meta-analysis 1						
MAT	602.85	13.01	589.84	0.002	<0.001	461
MAP	583.01	20.40	562.60	<0.001	<0.001	442
Duration	1090.24	0.88	1089.24	<0.001	0.35	872
Species richness	1063.63	5.01	1058.62	−0.012	0.03	872
In meta-analysis 2						
MAT	179.66	1.87	177.79	0.004	0.17	113
MAP	259.86	0.61	259.26	<0.001	0.44	188
Duration	368.56	0.04	368.51	<0.001	0.84	269
Initial carbon (C)	276.48	2.68	273.80	0.003	0.10	204
Initial nitrogen (N)	316.76	0.02	316.74	0.003	0.88	232
Initial phosphorus (P)	137.69	3.86	133.83	−0.896	0.05	100
Initial lignin (L)	220.03	0.14	219.90	0.001	0.71	167
Initial C/N	272.99	0.00	272.98	<0.001	0.99	204
Initial C/P	131.48	0.18	131.29	<0.001	0.67	95
Initial N/P	132.68	0.19	132.49	0.001	0.66	100
Initial N/L	218.42	0.07	218.34	−0.072	0.79	167
Initial P/L	51.77	0.42	51.34	14.735	0.52	41

Statistical results are reported as total heterogeneity in effect sizes among studies (Q_T), the difference among group cumulative effect sizes (Q_M) and the residual error (Q_E) from continuous randomized-effects model of meta-analyses. The relationship is significant at $P < 0.05$.

than the medium- and high-quality litter (Fig. 3d). When tree species were grouped into broadleaf/needle or evergreen/deciduous forms, the results consistently showed synergistic effects. Furthermore, the continuous randomized-effect model showed a significant positive correlation between litter initial P and the effect size of meta-analysis 2 (R_{sin} vs R_{mix}); however, the remaining initial nutrients of litter did not show any correlation with effect size (Table 1).

Discussion

The mixing effect across all studies

This study aimed to quantify the general effects of litter mixing on its decay rates. We showed that litter mixing often demonstrates nonadditive effects, and that these effects are most frequently synergistic, which is consistent with two previous literature reviews (Gartner & Cardon, 2004; Li *et al.*, 2016). Specifically, the decay rates of litter mixtures were, on average, 3–5% faster when compared to decay rates of single litter species (Fig. 2a). This significant but weak synergy makes sense in the context of the many forces that govern litter decomposition: when different litter species mix, many processes (including those that both stimulate and dampen litter decomposition) occur

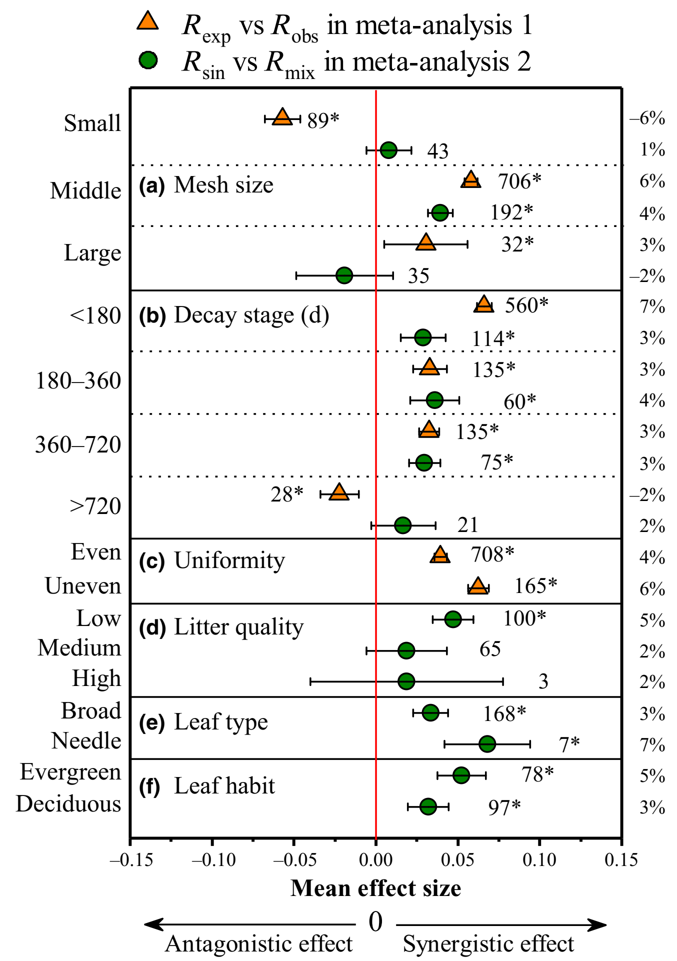

Fig. 3 Comparison of mixing litter decay rates between expected values (R_{exp}) and observed values (R_{obs}) (meta-analysis 1, orange triangles), and comparison of species-specific decay rates between single (R_{sin}) and mixture (R_{mix}) decomposition (meta-analysis 2, green circles) among different (a) mesh sizes, (b) experimental duration (days), (c) uniformity, (d) litter quality, (e) leaf types and (f) leaf habits. If the mean effect size > 0, then there is a synergistic effect; and if the mean effect size < 0, then there is an antagonistic effect. If the effect size 95% CIs (error bars) do not include zero, then the nonadditive effect was considered to be significant (*). The sample size for each variable is shown next to the point. The data on the right-hand *y*-axis represent the mean percentage difference for each variable (%).

simultaneously, with some factors probably counterbalancing others.

When the data were divided into different climatic zones, responses were variable, supporting Hypothesis 1. For example, an antagonistic effect of litter mixing was observed in the boreal zone, but a synergistic effect was observed in the temperate zone (meta-analyses 1 and 2; Figs 2b, 4b). A significant, positive correlation between MAT and its effect size on decomposition rate further supported this hypothesis (Table 1). This phenomenon probably resulted from: low soil fauna and microbial biomass in high- compared to low-latitude regions (Fierer *et al.*, 2009; Xu *et al.*, 2013; Nielsen *et al.*, 2014); soil organism activities may be limited by lower temperatures in high-latitude areas, lowering their activities and abundances, and preventing them from performing their well-documented function of accelerating litter decomposition (Pietikäinen *et al.*, 2005) and causing potential synergistic effects; and/or fungal hyphae growth in litter may be limited by low temperatures and precipitation in boreal zones, thereby impeding nutrient transport from the high- to the low-quality litter. Furthermore, when the data were classified into different ecosystems, shrublands showed an antagonistic effect (Fig. 2c), which could be because most of the shrubland data were acquired from boreal zones.

Invertebrate decomposers are key regulators of the mixing effect

In congruence with Hypothesis 2, we found that the presence of invertebrate decomposers influenced litter-mixing dynamics. Soil fauna (or invertebrate decomposers in aquatic systems) are an important group of decomposers, but they are difficult to directly control in the studies of litter decomposition. Litter bags with varying mesh sizes are often used to exclude different kinds of soil fauna. When fine mesh (<1 mm) litterbags were applied to exclude soil fauna in litter decomposition studies, an antagonistic effect of litter mixing on decomposition was detected (Figs 3a, 4b). Conversely, synergistic effects were detected when middle- (1–5 mm) or large-mesh (> 5 mm) litterbags were used. These results mirror the findings of another recent litter mixing study, which also found effects ranging from antagonism to synergism for litter mixtures (Barbe *et al.*, 2018); in that study, the direction of the litter mixture response was attributed to the phylogenetic and functional relatedness of the litter, with more phylogenetically diverse, functionally distinct litter mixtures exhibiting more synergistic decomposition responses. In our study, however, the phylogenetic and functional relatedness of litter mixtures were not considered, and these responses were attributed to the presence of litter fauna in the mixtures, which can modify consumer–resource interactions, leading to nonadditive mixing effects (Kominoski *et al.*, 2009; Sanper-Calbet *et al.*, 2009). Leaf litter rich in nutrients and energy can be more palatable to soil fauna than relatively lower quality leaf litter (Zhang *et al.*, 2016), which may accelerate the decay rate of litter mixtures. Another global synthesis on litter decomposition demonstrated that the decay rate of litter decreased by one-third when soil fauna were excluded (Zhang *et al.*, 2015). Additionally, soil fauna may make the litter more accessible to bacteria and fungi, which could stimulate microbial growth, thereby further promoting litter decay (Smith & Bradford, 2003).

An inexplicable pattern that emerged from our study was the nonsignificant antagonistic effect that occurred with the large-mesh litterbag treatment in meta-analysis 2. We hypothesize that this pattern could be due to a trophic cascade (*sensu* Terborgh & Estes, 2010; Sitvarin *et al.*, 2016), where large-mesh litterbags allow in larger predators from higher trophic levels that could reduce, either through predation or behavioral modification, the contribution of detritivores to litter decomposition, thereby weakening the synergistic effect of litter mixing on decomposition seen in medium-mesh litterbags. While testing this hypothesis is outside the scope of our study, our findings from both

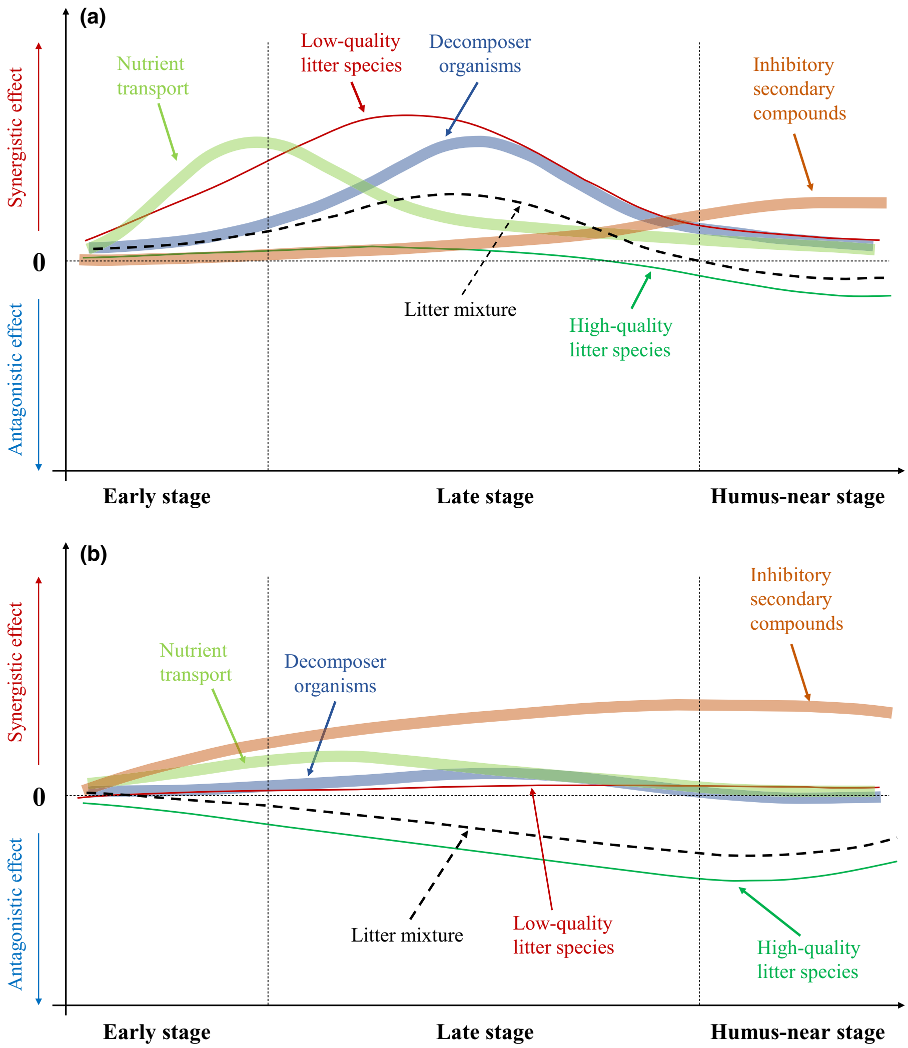

Fig. 4 Conceptual model for nonadditive effects in mixed litter decomposition of two species based on our meta-analyses. (a) The nonadditive effect in tropical and temperate areas. (b) The nonadditive effect in boreal areas or in the absence of soil fauna. The wide green, blue and brown lines represent the effect of nutrient transport, decomposer organisms and inhibitory secondary compounds on litter mixing effects, respectively. The fine green and red lines represent the response of high- and low-quality litter species on litter mixture effects, respectively. The black dashed line represents the overall litter mixing effect. The three stages of litter decomposition (early, late and near-humus stage) are referred to in Berg (2014). If the expected decay rate of the litter mixture is slower than its observed decay rate, then there is a synergistic effect; and if the expected decay rate of the litter mixture is faster than its observed decay rate, then there is antagonistic effect of litter mixing.

meta-analyses support this notion, as both analyses revealed that the medium-mesh litterbags yielded stronger synergistic effects compared to large-mesh litterbags, which either had a weaker synergistic effect (meta-analysis 1) or no effect (meta-analysis 2). That said, we caution against over-interpretation of our findings, especially given the small sample size for large-mesh litterbags in meta-analysis 2 ($n = 35$ observations), and encourage more researches to test this hypothesis and determine if this pattern is more widely generalizable.

That the synergistic effect of litter mixing weakens as a function of decomposition time (changing to an antagonistic effect after 720 d of decay) speaks to the role of invertebrate decomposers in mitigating how litter mixtures decompose, further corroborating our second hypothesis (Figs 3b, 4a). In the early stages of decomposition, the input of fresh litter provides abundant carbon and nutrients for soil fauna and microorganisms. In addition, studies suggest that nutrient transport frequently occurs in the early stages of decomposition (Hansson *et al.*, 2010; Liao *et al.*, 2016). Thus, large influxes of litter rich in nutrients, combined with high rates of nutrients diffusing from these substrates, may facilitate the growth and activities of soil microorganisms and ultimately produce a synergistic effect. Despite the role of soil fauna in promoting this synergistic effect, however, their relative role may be weakened and their effects may disappear at late stages of decomposition. Consequently, late-stage litter decomposition is probably performed primarily by microbes that can degrade very recalcitrant compounds (lignin, tannin, etc.) (Chapman *et al.*, 2013; Guénon *et al.*, 2017). Furthermore, recalcitrant compounds remaining in low-quality litter in late stages of decay probably contribute to the observed antagonistic effect (Fig. 4).

Litter mixture composition and species traits affect mixing effects

Both species evenness and richness influence the ecosystem functions, including litter decomposition (Dangles & Malmqvist, 2004; Tilman *et al.*, 2014). Although the evenness of species in litter mixtures has inconsistent effects on litter decomposition (Hillebrand *et al.*, 2008; Ward *et al.*, 2010; Li *et al.*, 2013), these meta-analyses indicate that the synergistic effect of uneven litter mixtures is higher than that of even mixtures, supporting Hypothesis 3 (Fig. 3c). We speculated that uneven litter mixtures, typical of natural systems, favor microbial growth more than even mixtures (Swan *et al*., 2009), but this requires further investigation. Our results also show a significant negative relationship between litter richness and its effect size on litter decomposition in meta-analysis 1 (Table 1). Other studies have shown that fungal diversity increases with litter richness (Otsing *et al*., 2018), but we still do not know how changes in associated fungal communities influence the decomposition of litter mixtures. Although the underlying reasons are unclear, we speculate that the following mechanism may contribute to this phenomenon: the rate of litter decomposition might have a threshold value in most systems (Fig. 4) (Barbe *et al*., 2018), whereby synergistic effects diminish after a certain number of species are present in the litter mixture. This could occur, for example, when the hyperniche for decomposers is maximized, such that adding new species does not add beneficial compounds or remove limiting resources. However, the relatively small size of our dataset does not afford us enough power to illuminate a global threshold or specific thresholds for different ecosystems.

In support of Hypothesis 4, we observed a synergistic effect of litter mixing for low-quality litter species, while only additive effects were observed in medium- and high-quality litter mixtures (meta-analysis 2; Figs 3d, 4a). Nutrients released from high-quality litter species promote low-quality litter decay (Versini *et al*., 2016; He *et al*., 2019), probably because these nutrients remove limits to the growth of soil organisms (Hättenschwiler & Jørgensen, 2010; Chapman *et al*., 2013), thus leading to a higher biomass of decomposers to do the work of decomposition, accelerating the decay of recalcitrant litter. Despite the fact that recalcitrant compounds that leach from low-quality litter could retard the decomposition of high-quality litter in mixtures (Gao *et al*., 2016), our results indicate that the magnitude of this effect is not great enough to counterbalance the benefit to detritivores from added nutrients provided by high-quality litter. Interestingly, the additivity observed in medium- and high-quality mixtures (Fig. 3d) suggests that the benefit of added nutrients from novel species in mixtures only applies to cases where low-quality litter is present, perhaps because these medium- and high-quality mixtures represent microhabitats that are not nutrient-limited. Still, the wide range of variability in the high-quality litter mixture response suggests that there is a lot we still do not understand about how litter quality influences decomposition dynamics in mixtures, and it is likely that factors other than nutrients become important when nutrient limitation is released.

In further support of Hypothesis 4, results based on litter type (i.e. broadleaf/needle, and evergreen/deciduous) showed that the mean effect size of needle and evergreen groups were higher than those of broadleaf and deciduous groups (Fig. 3e,f). In general, needle species contain more lignin than broadleaf species, and they will probably benefit more from the added nutrients from novel species in the mixture, leading to stronger synergistic effects of decomposition in litter mixtures. For this meta-analysis, mean lignin content of evergreen leaves was $26 \pm 8\%$, which was higher than that of deciduous leaves ($22 \pm 6\%$, Table S1). Moreover, across large spatial scales deciduous leaves can contain more N and P than evergreen leaves (Han *et al*., 2005), probably enhancing the decomposability of deciduous relative to evergreen leaves (Cornwell *et al*., 2008; Liu *et al*., 2016).

Conclusions

Based on our meta-analyses, we have developed a conceptual model of the nonadditive effect in mixed litter samples composed of two species (Fig. 4). It should be noted that the model reflects a general pattern, which may not be relevant to all cases. In tropical and temperate areas (Fig. 4a), litter mixing generally tends to cause a synergistic effect compared to single litters at the early and late decay stages. Nutrient transfer and the decomposer community were probably the primary drivers of these patterns. Moreover, when the litter mixtures were partitioned based on litter quality, low-quality litters display a synergistic decomposition effect, yet there was no change in medium- or high-quality litter species. At near-humus stages of decomposition, the release of inhibitory secondary compounds from low-quality litter probably dampens high-quality litter decay, and the synergistic effect changes to an antagonistic effect. In boreal areas (Fig. 4b), by contrast, antagonistic effects are predominant in litter-mixture decomposition dynamics, and the decay of high-quality litter species is markedly depressed by inhibitory secondary compounds or when soil fauna is absent. We suggest that synergistic and antagonistic effects, whose interplay yields nonadditive effects, occur simultaneously rather than independently. Collectively, our findings elucidate how key ecological factors govern decomposition dynamics in litter mixtures.

Acknowledgements

We thank all the authors of the original studies included in our meta-analyses. The study was supported by the National Natural Science Foundation of China (41703065, U1805243, 41807028, 31460077), the Natural Science Foundation of Fujian Science and Technology Department (2018J01621), and the Education and Research Projects for Young Teachers of Fujian Provincial Education Department (JAT170187). CJL was supported by US National Science Foundation grant DEB 1836387. We thank Shaofei Jin and Guiwu Zou for editing of graphs.

Author contributions

JL, YH and QY designed the study. JL conducted data checking. JL and QY conducted the analyses. JL and YH wrote the first draft of the manuscript. JL, HW, XL, QS, FL, YH and QY contributed to data collection, writing and revisions. ZC and CJL contributed data to the meta-analyses and contributed to all manuscript revisions.

ORCID

Zacchaeus G. Compson https://orcid.org/0000-0003-2015-3015
Carri J. LeRoy https://orcid.org/0000-0002-1185-4437

Jun Liu https://orcid.org/0000-0001-9311-1874

References

Ball BA, Hunter MD, Kominoski JS, Swan CM, Bradford MA. 2008. Consequences of non-random species loss for decomposition dynamics: Experimental evidence for additive and non-additive effects. *Journal of Ecology* **96**: 303–313.

Barbe L, Mony C, Jung V, Santonja M, Bartish I, Prinzing A. 2018. Functionally or phylogenetically distinct neighbours turn antagonism among decomposing litter species into synergy. *Journal of Ecology* **106**: 1401–1414.

Berg B. 2014. Decomposition patterns for foliar litter – a theory for influencing factors. *Soil Biology & Biochemistry* **78**: 222–232.

Bradford MA, Berg B, Maynard DS, Wieder WR, Wood SA. 2016. Understanding the dominant controls on litter decomposition. *Journal of Ecology* **104**: 229–238.

Chapman SK, Newman GS, Hart SC, Schweitzer JA, Koch GW. 2013. Leaf litter mixtures alter microbial community development: Mechanisms for non-additive effects in litter decomposition. *PLoS ONE* **8**: e62671.

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N *et al.* 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* **11**: 1065–1071.

Dangles O, Malmqvist B. 2004. Species richness–decomposition relationships depend on species dominance. *Ecology Letters* **7**: 395–402.

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC. 2009. Global patterns in belowground communities. *Ecology Letters* **12**: 1238–1249.

Gao J, Kang F, Han H. 2016. Effect of litter quality on leaf-litter decomposition in the context of home-field advantage and non-additive effects in temperate forests in China. *Polish Journal of Environmental Studies* **25**: 1911–1920.

Gartner TB, Cardon ZG. 2004. Decomposition dynamics in mixed-species leaf litter. *Oikos* **104**: 230–246.

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, Hättenschwiler S. 2010. Diversity meets decomposition. *Trends in Ecology and Evolution* **25**: 372–380.

Guénon R, Day TA, Velazco-Ayuso S, Gros R. 2017. Mixing of Aleppo pine and Holm oak litter increases biochemical diversity and alleviates N limitations of microbial activity. *Soil Biology & Biochemistry* **105**: 216–226.

Han W, Fang J, Guo D, Zhang Y. 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytologist* **168**: 377–385.

Hansson K, Kleja DB, Kalbitz K, Larsson H. 2010. Amounts of carbon mineralised and leached as DOC during decomposition of Norway spruce needles and fine roots. *Soil Biology & Biochemistry* **42**: 178–185.

Hättenschwiler S, Jørgensen HB. 2010. Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. *Journal of Ecology* **98**: 754–763.

Hättenschwiler S, Tiunov AV, Scheu S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics* **36**: 191–218.

He W, Ma Z, Pei J, Teng M, Zeng L, Yan Z, Huang Z, Zhou Z, Wang P, Xin Luo *et al.* 2019. Leaf litter decomposition in the Three Gorges Reservoir, China. *Forests* **10**: 360.

Hedges LV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* **80**: 1150–1156.

Hillebrand H, Bennett DM, Cadotte MW. 2008. Consequences of dominance: A review of evenness effects on local and regional ecosystem processes. *Ecology* **89**: 1510–1520.

Jabiol J, Chauvet E. 2015. Biodiversity and litter decomposition: a case study in a Mediterranean stream. *Freshwater Science* **34**: 423–430.

Knorr M, Frey S, Curtis P. 2005. Nitrogen additions and litter decomposition: a meta-analysis. *Ecology* **86**: 3252–3257.

Kominoski JS, Pringle CM, Ball BA, Bradford MA, Coleman DC, Hall DB, Hunter MD. 2007. Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology* **88**: 1167–1176.

Leroy F, Gogo S, Buttler A, Bragazza L, Laggoun-Défarge F. 2018. Litter decomposition in peatlands is promoted by mixed plants. *Journal of Soils and Sediments* **18**: 739–749.

Li D, Peng S, Chen B. 2013. The effects of leaf litter evenness on decomposition depend on which plant functional group is dominant. *Plant and Soil* **365**: 255–266.

Li YN, Zhou XM, Zhang NL, Ma KP. 2016. The research of mixed litter effects on litter decomposition in terrestrial ecosystems. *Acta Ecologica Sinica* **36**: 4977–4987.

Liao S, Ni X, Yang W, Li H, Wang B, Fu C, Xu Z, Tan B, Wu F. 2016. Water, rather than temperature, dominantly impacts how soil fauna affect dissolved carbon and nitrogen release from fresh litter during early litter decomposition. *Forests* **7**: 249.

Liu C, Liu Y, Guo K, Zhao H, Qiao X, Wang S, Zhang L, Cai X. 2016. Mixing litter from deciduous and evergreen trees enhances decomposition in a subtropical karst forest in southwestern China. *Soil Biology & Biochemistry* **101**: 44–54.

Madritch MD, Cardinale BJ. 2007. Impacts of tree species diversity on litter decomposition in northern temperate forests of Wisconsin, USA: a multi-site experiment along a latitudinal gradient. *Plant and Soil* **292**: 147–159.

Montané F, Romanya J, Rovira P, Casals P. 2013. Mixtures with grass litter may hasten shrub litter decomposition after shrub encroachment into mountain grasslands. *Plant and Soil* **368**: 459–469.

Nielsen UN, Ayres E, Wall DH, Li G, Bardgett RD, Wu T, Garey JR. 2014. Global-scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties. *Global Ecology and Biogeography* **23**: 968–978.

Otsing E, Barantal S, Anslan S, Koricheva J, Tedersoo L. 2018. Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter. *Soil Biology and Biochemistry* **125**: 328–339.

Pietikäinen J, Pettersson M, Bååth E. 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. *FEMS Microbiology Ecology* **52**: 49–58.

Rosenberg MS, Adams DC, Gurevitch J. 2000. *MetaWin: statistical software for meta-analysis.* Sunderland, MA, USA: Sinauer Associates Inc.

Sanper-Calbet I, Lecerf A, Chauvet E. 2009. Leaf diversity influences in-stream litter decomposition through effects on shredders. *Freshwater Biology* **54**: 1671–1682.

Schimel JP, Hattenschwiler S. 2007. Nitrogen transfer between decomposing leaves of different N status. *Soil Biology & Biochemistry* **39**: 1428–1436.

Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K. 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME Journal* **6**: 1749–1762.

Sitvarin MI, Rypstra AL, Harwood JD. 2016. Linking the green and brown worlds through nonconsumptive predator effects. *Oikos* **125**: 1057–1068.

Smith VC, Bradford MA. 2003. Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. *Applied Soil Ecology* **24**: 197–203.

Steinwandter M, Schlick-Steiner BC, Steiner FM, Seeber J. 2019. One plus one is greater than two: mixing litter types accelerates decomposition of low-quality alpine dwarf shrub litter. *Plant and Soil* **438**: 405–419.

Swan CM, Gluth MA, Horne CL. 2009. Leaf litter species evenness influences nonadditive breakdown in a headwater stream. *Ecology* **90**: 1650–1658.

Terborgh J, Estes JA. 2010. *Trophic cascades: predators, prey, and the changing dynamics of nature.* Washington, DC, USA: Island Press.

Tilman D, Isbell F, Cowles JM. 2014. Biodiversity and ecosystem functioning. *Annual Review of Ecology, Evolution, and Systematics* **45**: 471–493.

Tiunov AV. 2009. Particle size alters litter diversity effects on decomposition. *Soil Biology & Biochemistry* **41**: 176–178.

Versini A, Laclau JP, Mareschal L, Plassard C, Diamesso LA, Ranger J, Zeller B. 2016. Nitrogen dynamics within and between decomposing leaves, bark and branches in *Eucalyptus* planted forests. *Soil Biology & Biochemistry* **101**: 55–64.

Ward SE, Ostle NJ, McNamara NP, Bardgett RDJO. 2010. Litter evenness influences short-term peatland decomposition processes. *Oecologia* **164**: 511–520.

Wiebe N, Vandermeer B, Platt RW, Klassen TP, Moher D, Barrowman NJ. 2006. A systematic review identifies a lack of standardization in methods for handling missing variance data. *Journal of Clinical Epidemiology* **59**: 342–353.

Xu X, Thornton PE, Post WM. 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Global Ecology and Biogeography* **22**: 737–749.

Zhang D, Hui D, Luo Y, Zhou G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology* **1**: 85–93.

Zhang W, Chao L, Yang Q, Wang Q, Fang Y, Wang S. 2016. Litter quality mediated nitrogen effect on plant litter decomposition regardless of soil fauna presence. *Ecology* **97**: 2834–2843.

Zhang WD, Yuan SF, Hu N, Lou YL, Wang SL. 2015. Predicting soil fauna effect on plant litter decomposition by using boosted regression trees. *Soil Biology & Biochemistry* **82**: 81–86.

Zhao W, Logtestijn RSPV, Hal JRV, Dong M, Cornelissen JHC. 2019. Non-additive effects of leaf and twig mixtures from different tree species on experimental litter-bed flammability. *Plant and Soil* **436**: 311–324.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 The nonadditive effect in mixed litter decomposition. The nonadditive effect includes synergistic effects (green triangles) and antagonistic effects (yellow triangles).

Fig. S2 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram showing the process of determining which studies to include in these meta-analyses.

Fig. S3 World-wide distribution of selected studies in these meta-analyses.

Notes S1 List of all the references used in these meta-analyses.

Table S1 Summary of the references and data used in these meta-analyses of the effects of litter mixing on decomposition rate.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.