# Synergistic effect: a common theme in mixed-species litter decomposition

Jun Liu[1, 2] https://orcid.org/0000-0001-9311-1874, Xiaoyu Liu[2], Qingni Song[1], Fenggang Luan[1], Wang Hui[3], Yalin Hu[2*], Qingpei Yang[1*]

[1]*Jiangxi Provincial Key Laboratory for Bamboo Germplasm Resources and Utilization, Jiangxi Agricultural University, Nanchang 330045, China*

[2]*Stable Isotope Center, College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, China*

[3]*College of Resources and Environment, Shandong Agricultural University, Taian 271018, China*

*Author for correspondence:

*Yalin Hu,*

*E-mails: huyl@iae.ac.cn*

*Qingpei Yang*

*E-mails: qingpeiyang@126.com*

## Abstract

- Litter decomposition plays a key role in terrestrial ecosystem nutrient cycling; yet, to date, science is lacking a comprehensive understanding of the non-additive effect in mixing litter decomposition.
- In order to help fill that gap, we compiled 63 individual studies for the purpose of performing a meta-analysis of the non-additive effect in terrestrial ecosystems.
- Results indicate that a synergistic effect: 1) dominates in mixed litter decay with an average increase of +3% over single litter decay; 2) occurs during the early and medium decomposition stages; 3) increases with mean air temperature and decreases with latitude; and 4) is observed in separated mixing litter studies with low-quality species, while no significant change is observed with high-quality species.
- Our meta-analysis provides a systematic evaluation of the non-additive effect in terrestrial ecosystems' mixing litter decomposition, which is critical for understanding and improving carbon forecasts and nutrient dynamics.



## Keywords

Non-additive effect, litter mixture, litter quality, synergistic effect, litter decomposition, meta-analysis


## Introduction

Litter decomposition is a central component of biogeochemical cycles in terrestrial ecosystems; as it controls nutrient return and energy flow, which regulates atmospheric carbon emissions, soil organic matter composition, and the mineral based nutrient supply to flora. Thus, litter decomposition influences ecosystem primary productivity (Bradford *et al.,* 2016). Over the past few decades, most litter decomposition studies have focused on single litter decay (Gartner & Cardon, 2004), which resulted in a profound exploration of the connection between decomposition rate and its impact factors. However, in most terrestrial ecosystems, litter is generally a mix of multiple species. Previous studies suggest that the various litter species interact with each other during litter decomposition (Gessner *et al.,* 2010), which implies that discerning the impact of litter mixture on litter decomposition is essential to understanding carbon and nutrient cycles.

When different litter species are mixed together, the decomposition rates generally do not equal the arithmetic mean value, i.e., the expected decay rate, between single litter species (Fig. 1) (Gartner & Cardon, 2004; Steinwandter *et al.,* 2019). When different litter species are mixed together, the decomposition rates generally do not equal the arithmetic mean value, i.e., the expected decay rate, between single litter species (Fig. 1) (Gartner & Cardon, 2004; Steinwandter et al., 2019).

Instead, one of two potential results usually develops: synergistic effect and antagonistic effect (Fig. 1). In general, decomposer activity, chemical characteristics of litter, and environmental factor constrains decomposition dynamics. In litter mixture, nutrients (such as nitrogen) transfer from high-quality litter species to low-quality litter species, complementary effects on fauna and decomposers, and improvement of microclimatic conditions are regarded as formation mechanisms of synergistic effect (Schimel & Hattenschwiler, 2007; Tiunov, 2009 Madritch and Cardinale 2007). Conversely, the antagonistic effect may induced by enhancement of microbial nutrient immobilization of nutrient poor litter or inhibitory secondary compounds released from low-quality litter species (Hättenschwiler et al., 2005, Montané 2013).

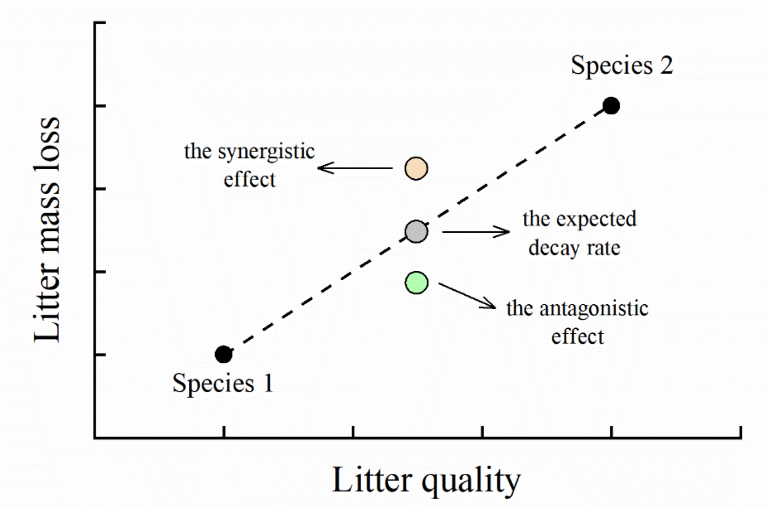


**Figure 1** The non-additive effect in mixing litter decomposition. The Grey circle represents the expected decay rate (Rexp) after litter mixing, Rexp = $w_1R_1 + w_2R_2 + \ldots + wnRn$, where wn is the weight of species n in the mixture and Rn is the decomposition rate of species n. The additive effect and non-additive effect are when the observed and expected decay rates are equal and not equal, respectively. The non-additive effect includs the synergistic effect (yellow circle) and antagonistic effect (green circle).

While experiments concerning litter mixture effects on decomposition rates have been conducted in numerous individual studies, the conflicting results have hampered any possibility of drawing general conclusions. Much of the conflict has possibly resulted from significant differences in experimental design. The various studies conducted thus far were all performed in different climate regions and ecosystems. In addition, decomposition experiment durations varied from several weeks to a number of years. Moreover, while litterbag and microcosm were the most commonly employed methods, different mesh sizes were used, making it unclear whether the results are comparable. As such, for the past several years, the scientific community has been requesting a meta-analysis in order to determine a general global pattern (Gessner *et al.* 2010).

Although studies throughout the literature have summarized and analyzed the non-additive effect in mixed litter decomposition (Gartner & Cardon, 2004; Li *et al.,* 2016), there still remains a lot of unanswered questions. In order to better understand how does mixing influence decomposition rate, we employed a meta-analysis that built upon the system analysis done by Gartner and Cardon (2004). Finally, 63 individual studies were compiled to perform a global analysis (Note S1). If litter mixing effect occur, we also aimed to explore eight follow detailed questions:

1) How does climate influence the effect of mixing on decomposition?
2) How does biome influence the effect of mixing on decomposition?
3) How does litterbag mesh size influence the effect of mixing on decomposition?
4) How does length of experiment influence the effect of mixing on decomposition?
5) How does evenness and species number of mixing influence the effect of mixing on decomposition?
6) How does lignin content (AKA litter quality) influence the effect of mixing on decomposition?
7) How does leaf type influence the effect of mixing on decomposition?
8) How does habit influence the effect of mixing on decomposition?

## Materials and Methods

### Data compilation

Publications that reported data on litter mixture decomposition were selected from the Web of Science resource, Google Scholar, and the China National Knowledge Infrastructure. Keyword search strings consisted of term combinations, such as (litter or debris or residues) AND (mix* or diversity or non-additive effect or additive effect) AND (decompos* or decay* or degrad*). The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram performed the procedure used for selection of studies for meta-analysis (Fig S1).

Any selected publication had to satisfy the following three criteria: 1) it had to report at least one of our selected variables [(expected decay values (Rexp) vs. observed decay values (Robs)], or species-specific decay rates in mixture vs. in unmixed); 2) it had to provide the means and sample sizes (n) of the variables selected for the meta-analysis or could be calculated from the chosen papers; 3)the measurements of selected variables were performed at the same temporal and spatial scales; and 4) expect litter mixture treatment, the study under other experimental treatments (such as nutrient addition, warming, water controlling) was excluded. If the data were presented in figures, Getdata Graph Digitizer (version 2.24) was used to extract the numerical values. In addition, we sent Emails to corresponding authors to query the original data that k values ($yr^{-1}$) or relative mixture effect [(observed mass loss- expected mass loss)/ expected mass loss*100] was used to estimate the non-additive effect in papers.

We separate the dataset into two parts to conduct two sub-meta-analyses (Fig. 2). The first sub-meta-analysis was compared mixing litter decay rates between expected decay rates (Rexp) and observed decay rates (Robs), and the second sub-meta-analysis was compared species-specific decay rates between single (Rsin) and mixture (Rmix) decomposition. Specially, the questions 6-8 were only answered by the second sub-meta-analysis.

In total, 513 pair observations of expected decay rate versus observed decay rate were obtained from 47 selected papers and 258 pair observations of single litter decomposition treatment versus mixing litter decomposition treatment on single litter decay rate were obtained from 16 selected papers. The studies were primarily conducted in East Asia, Western Europe, and North America (Fig. S2).

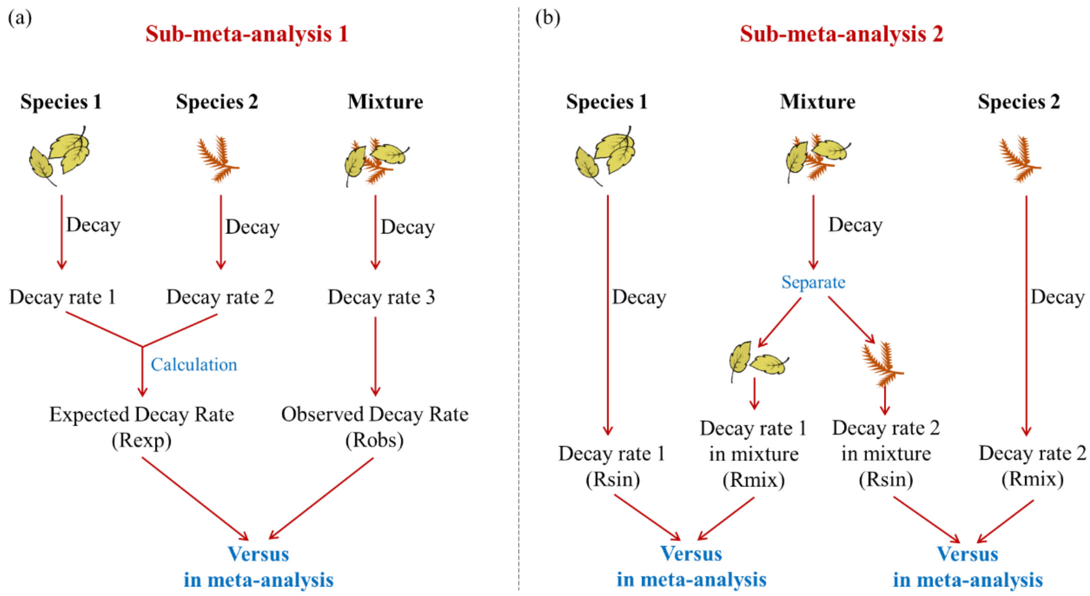


**Figure 2** The two sub-meta-analyses in this study. (a) the sub-meta-analysis 1, comparison of mixing litter decay rate between expected values (Rexp) and observed values (Robs); (b) the sub-meta-analysis 2, comparison of species-specific decay rate between in single (Rsin) and in mixture (Rmix) decomposition.

For each study, the site location, ecosystem type, species (and its nutrient contents if provided), decomposition duration, mesh size, response variables, and other background information (e.g. mean annual temperatures, mean annual precipitation, etc.) was noted.

## 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 litter mixture impacts on decomposition rate:

$$\ln R = \ln(\overline{Xt}/\overline{Xc}) = \ln(\overline{Xt}) - \ln(\overline{Xc}) \qquad (1)$$

where $\overline{Xt}$ and $\overline{Xc}$ represent the observed decomposition rate (Robs) and the expected decomposition rate (Rexp), respectively; or the single litter decay rate in mixing litter decomposition (Rmix) and single litter decomposition treatment (Rsin), respectively.

The variance of ln$R$ ($v$) was approximated using the following formula:

$$v = \frac{S_t^2}{n_t \overline{X}_t^2} + \frac{S_c^2}{n_c \overline{X}_c^2} \qquad (2)$$

where $S_t$ and $S_c$ are the standard deviations (SDs) for the Robs and Rexp, respectively, or for the single litter decay rate in mixing litter decomposition and single litter decomposition treatment, respectively; $n_t$ and $n_c$ are the sample sizes for the Robs and EDR respectively, or for the single litter decay rate in mixing litter decomposition and single litter decomposition treatment, respectively; If both the SD and standard error

(SE) were lacking, we multiplied the average coefficient of variation (CV) calculated from each data set by the reported mean to estimate the missing SD (Wiebe *et al.,* 2006).

A nonparametric weighting function was used to weight individual studies; and the mean effect size ($\overline{\ln R'}$) of all observations was estimated according to Eq. 3:

$$\overline{\ln R} = \frac{\sum_i \ln R_i}{\sum_i w_i} \qquad (3)$$

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

To determine whether a significant difference was observed under litter mixture treatment (Rosenberg *et al.,* 2000), we employed a fixed-effect model, using the Metawin 2.1 software, to calculate 95% confidence intervals (CIs) of the weighted effect size. The effect was only considered significant if the 95% CI values did not overlap with 0. Furthermore, to clearly express the non-additive effects, the mean effect size was converted back to percent changed, using the following equation:

$$(e^{\overline{\ln R_i}} - 1) \times 100\% \qquad (4)$$

In order to better understand the formation mechanisms of the non-additive effects, we grouped the data according to climate (tropical, temperate, frigid), ecosystem type (forest, shrubland, grassland, aquatic, peatland), mesh size [small (diameter < 1 mm), medium (1mm ⩽ diameter < 5) and large (diameter ⩾ 5mm)], and experiment duration (< 180 days, 180-360 days, 360-720 days, and >720 days). Finally, we also grouped trees and shrubs based on different functional types (broadleaf, needle, evergreen, and deciduous).

A continuous randomized-effect model was used to assess the potential linearity or non-linearity between lnR and environmental (mean annual precipitation (MAP) and mean air temperature (MAT)) or forcing factors (initial nutrients content, and experiment duration). The total ln$R'$ 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 on all the studies was significantly positive both in Rexp vs. Robs and Rsin vs. Rmix, with an average increase of +3% and 4%, respectively (Fig. 3a). When different climates were considered, mixing litter decomposition caused a significant positive response in temperate areas and a significant negative response in frigid areas in both of two sub-meta-analyses (Fig. 3b). In tropical area, Rexp was significantly higher than Robs, and no significant change between Rsin and Rmix. The continuous randomized-effect model indicated that MAT had a significant positive correlation with the mean effect size of Rexp vs. Robs and Rsin vs. Rmix (Table 1). Except shrubland showed a significant negative response in Rexp vs. Robs (mean effect size = -0.0849; 95% CI: -0.0972 ~ -0.0726), the mean effect sizes of litter mixing on decomposition rate were positive (Fig. 3c). There were only seven pair observations in peatland, too little sample for statistically significant analysis.

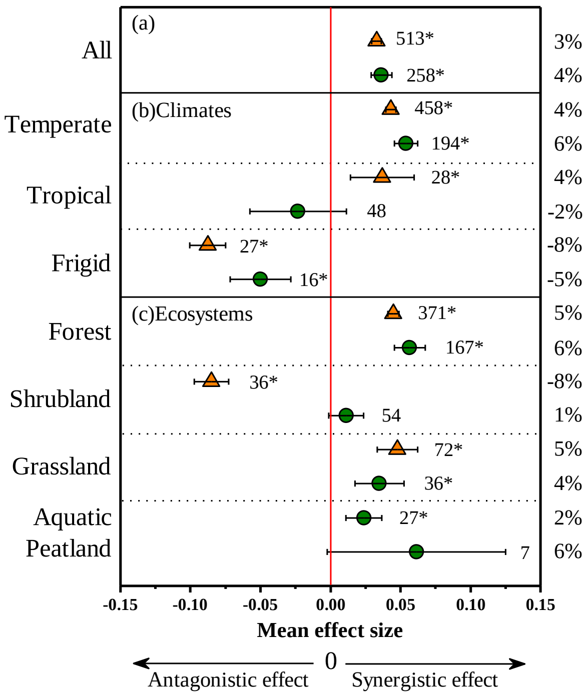


**Figure 3** Comparison of mixing litter decay rates between expected values (Rexp) and observed values (Robs) (sub-meta-analysis 1, orange triangles), and comparison of species-specific decay rates between in single (Rsin) and in mixture (Rmix) decomposition (sub-meta-analysis 2, green circles) (a) across all studies, (b) among different climates, (c) ecosystems. If the mean effect size = 0, then means additive effect ; If the mean effect size > 0, then means synergistic effect; If the mean effect size < 0, then means antagonistic effect. If the effect size 95% CI (error bars) did not cover zero, the non-additive effect was considered to be significant (*). The sample size for each variable is shown next to the point.

Varying experimental methods showed different effects on the decomposition rate (Fig. 4). Mesh size was divided into three groups—small, middle, and large—and it was determined that a significant antagonistic effect was found in small mesh size studies in Rexp vs. Robs. In contrast, the decomposition rate in the middle and large mesh size studies showed a significant synergistic response (Fig. 4a). For Rsin vs. Rmix, small and large mesh sizes depicted an additive effect, whereas, middle mesh size studies showed a +6% percent increase in decomposition rate (Fig. 4a).

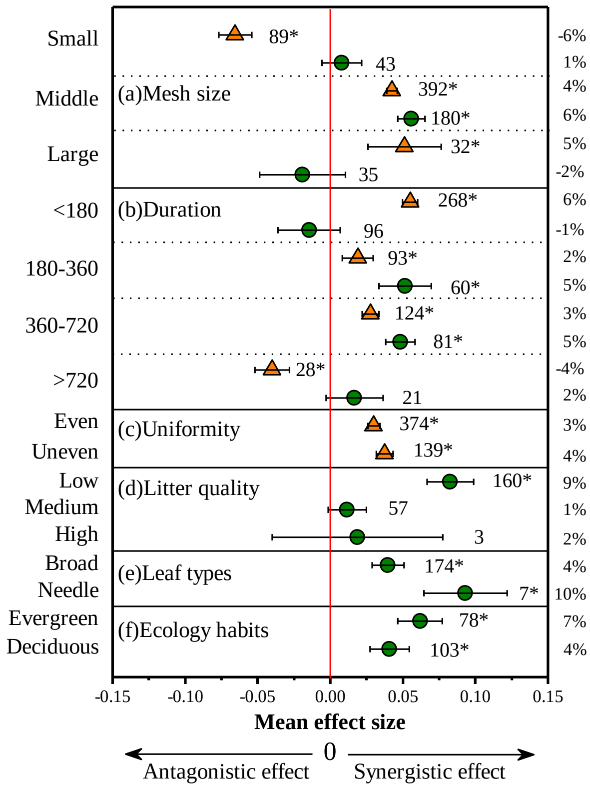


**Figure 4** Comparison of mixing litter decay rates between expected values (Rexp) and observed values (Robs) (sub-meta-analysis 1, orange triangles), and comparison of species-specific decay rates between in single (Rsin) and in mixture (Rmix) decomposition (sub-meta-analysis 2, green circles) among different experimental methods. (a) mesh size, (b) duration, (c)uniformity, (d) litter quality, (e) leaf types, and (f) ecology habits. If the mean effect size > 0, then means synergistic effect; If the mean effect size < 0, then means antagonistic effect. If the effect size 95% CI (error bars) did not cover zero, the non-additive effect was considered to be significant (*). The sample size for each variable is shown next to the point.

When experiment duration was partitioned into four levels, results showed that the decomposition rate increase was highest under short-term duration (<180 days, +6%), and a significant antagonistic effect was observed under long-term duration (>720 days; -4%) in Rexp vs. Robs (Fig. 4b). The continuous randomized-effect model also showed that decomposition duration had a significant negative correlation with the effect size (Table 1). The results showed an additive effect in the short-term period, i.e., < 180 days in Rsin vs. Rmix (Fig. 4b). On the other hand, significant synergistic response occurred during the mid-term period (180-720 days) and a marginally increasing trend during the long-term period (>720 days). In sub-meta-analysis of Rexp vs. Robs, both of even litter mixtures and uneven litter mixtures showed synergistic effects on decomposition rate (Fig. 4c).

In sub-meta-analysis of Rsin vs. Rmix, the studies were divided into three quality levels based on litter lignin content—low, medium, and high—and the results showed that the low-quality litter decomposition rate exhibits the largest change (Fig. 4d). Similarly, when tree species were grouped into broad/needle or evergreen/deciduous, results consistently showed synergistic effects.

The continuous randomized-effect model meta-analyses showed a significant positive correlation between MAT and the effect size of the sub-meta-analysis 1 (Rexp vs. Robs) (Table 1). Litters with initial C and initial lignin content also exhibited a significant positive correlation with the effect size of the sub-meta-analysis 2 (Rsin vs. Rmix) yet the remaining litter initial nutrient indexes did not show any correlation (Table 1).

**Table 1** Relationships between the mixing litter treatment effect size on decay rate and mean air temperature (MAT), mean annual precipitation (MAP), experiment duration, number of species, and litter initial nutrients content.

| | $Q_T$ | $Q_M$ | $Q_E$ | Slope | *P*-value |
|---|---|---|---|---|---|
| **Observation vs Prediction** | | | | | |
| **MAT** | **615.644** | **25.660** | **589.984** | **0.004** | **<0.001** |
| MAP | 584.229 | 24.474 | 559.755 | <0.001 | <0.001 |
| During days | 777.618 | 26.686 | 750.932 | -0.0001 | <0.001 |
| Species numbers | 733.038 | 1.176 | 731.862 | 0.009 | 0.278 |
| **Individual vs In mixture** | | | | | |
| MAT | 217.415 | 6.278 | 211.137 | 0.004 | 0.012 |
| MAP | 253.306 | 0.853 | 252.453 | <0.001 | 0.356 |
| During days | 338.254 | 0.029 | 338.226 | <0.001 | 0.865 |
| **Initial C** | **274.997** | **4.661** | **270.336** | **0.003** | **0.031** |
| Initial N | 299.500 | 0.289 | 299.211 | -0.010 | 0.591 |
| Initial P | 113.234 | 1.050 | 112.183 | -0.542 | 0.305 |
| **Initial Lignin** | **215.424** | **3.069** | **212.355** | **0.002** | **0.080** |
| Initial C/N | 268.603 | 0.117 | 268.486 | <0.001 | 0.733 |
| Initial C/P | 110.488 | 0.899 | 109.589 | <0.001 | 0.343 |
| Initial N/P | 111.668 | 0.209 | 111.459 | 0.001 | 0.647 |
| Initial N/L | 208.551 | 0.340 | 208.212 | -0.124 | 0.560 |
| Initial P/L | 42.720 | 1.055 | 41.665 | 18.542 | 0.304 |

Statistical results were reported as total heterogeneity in effect sizes among studies (QT), the difference among group cumulative effect sizes (QM), and the residual error (QE) from continuous randomized- effects model meta- analyses. The relationship is significant when $P < 0.05$.

## Discussion

### The non-additive effect across all studies

The primary goal of this study was to calculate the general effect of litter mixture on litter decay rates. Our results showed that litter mixture widely demonstrates a non-additive effect, and most frequently synergistic effect, which is consistent with two previous review studies (Gartner & Cardon 2004; Li *et al.,* 2016). The results imply that litter mixture decay rates are on average 3%-4% faster when compared to the decay rates of single litter species (Fig. 1a). This significant synergy is weak but logical. When different litter species mixing, many processes (include stimulating and restraining processes) may occurred simultaneity, each one more or less counterbalancing the others.

Litter decomposer (include microorganisms and soil fauna) variation play an important role in non-additive effect. While this meta-analysis is lacking sufficient data to discuss decomposer variation, a limited set of data (mean effect size = 0.1604; 95% CI: 0.1432~0.1776) demonstrated that microbial biomass is significantly higher in mixing litter decomposition, as compared to single litter decomposition. In litter mixture decomposition, high quality litter bring in more available carbon to promote microbes growth (Hättenschwiler and Jørgensen 2010), and extracellular enzyme activity also increased simultaneously (Hu et al. 2006), both of which can help low quality litter decay. The microbial biomass increase is believed to be an important cause for the generation of a synergistic effect.

When the data was divided into different climatic zones, an antagonistic effect was observed in frigid areas (Fig.3b, answer to Q1). This phenomenon likely resulted from 1) soil fauna and microbial biomass being low in high latitude regions compared with low latitude regions (Fierer *et al.,* 2009; Xu *et al.* 2013; Nielsen *et al.* 2014); and 2) the soil organisms' activity being limited by lower temperatures in high latitude areas. As stated above, soil fauna should be regarded as an important propelling force for the synergistic effect, and when their quantity and activity is substantially reduced, a synergistic effect seems unable to develop. Furthermore, a significant positive correlation between MAT and the effect size on decomposition rate also supports this hypothesis. When the data were classified into different ecosystems, an antagonistic effect was observed in shrubland (Fig.3c, answer to Q2); and most of the shrubland data were acquired from frigid areas.

Soil fauna is an important group of decomposers that is difficult to directly control in litter decomposition studies. Mesh diameter is usually used to distinguish different kinds of soil fauna. While a fine mesh (<1mm) was applied in order to exclude most of the soil fauna, the antagonistic effect plays a leading role (Fig. 4a, answer to Q3). Interestingly, the antagonistic effect changed into a synergistic effect when middle (1-5 mm) or coarse mesh (>5 mm) was used, suggesting that soil fauna should be acknowledged as an indispensable factor in the synergistic effect. High-quality litter with more nutrients and energy can be palatable to soil fauna (Zhang et al. 2016), which may accelerate litter mixture decay rate. A global synthesis studies also suggested that litter decay rate fall by a third if soil fauna excluded (Zhang et al. 2015). Therefore, the existence of high-quality litter in mixing litter decomposition can promote litter decay rate. In addition, soil fauna may benefit the litter more accessible to bacteria and fungi, which could stimulate microbial growth and therefore decomposition further (Smith and Bradford 2003).

It is worth noting that the synergistic effect gradually weakened as a function of decomposition time and turned into an antagonistic effect after 720 days of decay (Fig. 4b, answer to Q4). In the early decomposition stages, fresh litter input provides abundant food for the soil fauna and microorganisms. In addition, studies suggest that nutrient transport often occurs in the early decay stages (Hansson et al., 2010; Liao et al., 2016). Thus, the abundant food and efficient nutrient transport facilitate growth and activity of soil microorganisms, which ultimately produce a synergistic effect. As state before, fauna effects is important to the synergistic effect. Whereas, the role of fauna is less important and fauna effects maybe disappear at late stages of decomposition, and decomposition is mainly performed by microbes able to degrade very recalcitrant compounds. On the other hand, the remaining recalcitrant substances (such as lignin, tannin, etc) from low-quality species may resist degradation at later decay stages and subsequently generate an antagonistic effect.

Both species evenness and richness influence ecosystem functions, including decomposition processes (Dangles & Malmqvist 2004; Tilman et al., 2014). Although litter evenness depicted inconsistent effects on decomposition (Hillebrand et al., 2008; Ward et al., 2010; Li et al., 2013), this meta-analysis indicates that the synergistic effect in uneven litter mixtures is slightly higher than in even mixtures (Fig. 4c, answer to Q5). It is generally accepted that ecosystem primary productivity improves with plant richness (Liang et al., 2016; Duffy et al., 2017), and standing litter pools could develop, simultaneously. Our results show no significant relationship between plant richness and mixing litter decomposition rate (Table 1), indicating that nutrients would not return to the soil timely, which may lead to negative feedback effects on productivity (Knops et al., 2001; Duffy et al., 2017).

In sub-meta-analyses 2, although the separation of mixture litter components is laborious and expensive, it is indispensable for exploring the non-additive effect internal mechanisms (Gartner & Cardon 2004). Thus, several studies have devoted substantial, time, effort, and resources into furthering this investigation. A synergistic effect appeared when compared single litter decomposition in in single (Rsin) and in mixture (Rmix) decomposition. Thus, again the synergistic effect appears to dominate the non-additive effect.

With respect to the three classifications of litter species, a noteworthy phenomenon is that a significant synergistic effect was observed in low-quality litter species, while no significant change was detected in medium and high-quality litter species (Fig. 4d, answer to Q6). There are five possible combinations of two species litter under the synergistic effect (Table 2). Based on our results, the third possibility is the most likely scenario. Although the underlying reasons are unclear, we speculate that the following three factors may contribute to this result. First, the nutrients released from high-quality litter species promote low-quality litter decay. Second, the input of high-quality litter species promotes the growth of soil organisms; thus low-quality litter may become competitive leading to accelerated decomposition. Third, the improved microenvironment through the addition of high-quality litter input, facilitates low-quality litter decomposition (He *et al.,* 2019).

**Table 2** Possible combinations of two separate litter species of decomposition rate change

| **Litter species** | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| Low quality | ↑ | ↑ | ↑ | ↓ | - |
| High quality | ↑ | ↓ | - | ↑ | ↑ |

The "↑"means a significant synergistic effect, the "↓" means a significant antagonistic effect, and the "-" means no significant change.

Trees and shrubs were classified into four groups consisting of broad, needle, evergreen, and deciduous. Results showed that the mean effect size of needle and evergreen groups was higher than those of broad and deciduous groups (Fig. 4e, answer to Q7). In general, the needle contains more lignin than broadleaf. Studies also suggest that deciduous leaf decomposability is higher than that of the evergreen leaf (Cornwell *et al.,* 2008; Liu *et al.,* 2016). This data indicates that low-quality litter species decomposition is more sensitive to litter mixing treatment, which is in agreement with previously discussed experimental results (Fig. 4f, answer to Q8).

## Conclusions

In this work, we developed a conceptual model of the non-additive effect in mixed litter samples, composed of two species, based on our meta-analysis results (Fig. 7). It should be noted that the model reflects a general pattern, which may not be appropriate in all cases. Furthermore, additional studies are needed to perfect both the theory and the model. In summary, mixing litter decomposition generally increased the decay rate and tended to cause a synergistic effect. Moreover, when the mixing litter was separated, low quality-litter species displayed a synergistic effect, yet there was no change in high-quality litter species. Soil organisms, especially soil fauna, were regarded as important factors in generating a synergistic effect. A synergistic effect usually occurred at the early and late decay stages and disappeared at the humus-near stage. We suggest that the synergistic effect and antagonistic effect are not isolated occurrences, but instead occur simultaneously, and the non-additive effect results from a balance of the interplay between them. Our study provides a comprehensive overview of mixing litter decomposition's non-additive effect, which is of great significance for studies on nutrients cycles, species invasion, and sustainable forest management, etc.

## Acknowledgements

We thank all the authors of the original studies included in our meta-analysis. The study was supported by the National Natural Science Foundation of China (41703065, U1805243, 41807028), 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).

**Author contribution**

J. L, Y. H and Q. Y. designed the study. J. L conducted data checking. J. L conducted the analyses. J. L and Y. H wrote the first draft of the manuscript. J. L., Y. H., X. L., Q. S., F. L., Y. H.. contributed to data collection, writing, and revision.

**Supporting Information**

**Figure S1** The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram performed the procedure used for selection of studies for meta-analysis

**Figure S2** World distribution of selected studies in this meta-analysis.

**Note S1** List of all the references used in the meta-analysis.

**References**

**Berg B. 2014.** Decomposition patterns for foliar litter – A theory for influencing factors. *Soil Biology and 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.

**Duffy JE, Godwin CM, Cardinale, BJ. 2017.** Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature* **549**: 261-264.

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

**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. 2010.** Diversity meets decomposition. *Trends in Ecology and Evolution* **25**: 372-380.

**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 and Biochemistry* **42**: 178-185.

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

191-218.

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

**He W, Ma Z, Pei J, Teng M, Zeng L, Yan Z, Huang Z, Zhou Z, Wang P, Luo Xin *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.

**Hu, Y. L., S. L. Wang, and D. H. Zeng. 2006.** Effects of Single Chinese Fir and Mixed Leaf Litters on Soil Chemical, Microbial Properties and Soil Enzyme Activities. Plant and Soil **282**:379-386.

**Knops JMH, Wedin D, Tilman, D. 2001.** Biodiversity and decomposition in experimental grassland ecosystems. *Oecologia* **126**: 429-433.

**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.

**Liang J, Crowther TW, Picard N, Wiser S, Zhou M, Alberti G, Schulze ED, McGuire AD, Bozzato, F, Pretzsch H *et al*. 2016.** Positive biodiversity-productivity relationship predominant in global forests. *Science* **354**: aaf8957.

**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.

**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.

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

**Santonja M, Rancon A, Fromin N, Baldy V, Hättenschwiler S, Fernandez C, Mont ès N, Mirleau P. 2017.** Plant litter diversity increases microbial abundance, fungal diversity, and carbon and nitrogen cycling in a Mediterranean shrubland. *Soil Biology & Biochemistry* **111**: 124-134.

**Santschi F, Gounand I, Harvey E, Altermatt F. 2018.** Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. *Functional Ecology* **32**: 522-532.

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

**Smith, V. C., and M. A. Bradford. 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

**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.

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

**Zhang, W. D., S. F. Yuan, N. Hu, Y. L. Lou, and S. L. Wang. 2015.** Predicting soil fauna effect on plant litter decomposition by using boosted regression trees. Soil Biology & Biochemistry **82**:81-86.