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Soil fauna accelerate litter mixture decomposition globally, especially in dry environments

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Abstract
1. More than half of the net primary production in terrestrial ecosystems returns to the soil through leaf litter fall and decomposition. In terrestrial ecosystems, litter constitutes a mixture of mainly senescent foliage from multiple species. Yet, the effect of litter mixing on litter decomposition rate remains ambiguous. Quantification of the soil fauna contribution and inclusion of their interaction with litter could remove the prevailing ambiguity, as soil fauna might influence the direction and magnitude of litter mixture effects on decomposition.

2. We carried out a global meta-analysis to elucidate how soil fauna influenced litter mixture decomposition rate based on 55 published studies with 873 fauna inclusion/exclusion observations in field litterbag experiments. We hypothesized that soil fauna inclusion in litter mixture experiments would cause a positive change in the magnitude of mixed litter decomposition. That is, the presence of soil fauna would lead to synergistic litter mixture effects while the absence of soil fauna would lead to antagonistic litter mixture effects. Furthermore, we hypothesized that soil fauna would have a greater positive impact on the litter mixture effect in environments with low precipitation.

3. While litter mixture had a neutral effect on decomposition on average across studies, non-additive mixture effects were common, with a key role for soil fauna, which generally enhanced decomposition rate compared to the component single-species litters. In contrast, fauna exclusion resulted in diminished decomposition rate overall. The overall synergistic soil fauna effect on litter mixture decomposition increased under low precipitation. Under high precipitation, the litter mixture effect was positive in the absence of soil fauna but non-significant in the presence of fauna. Climate imposes a great effect on litter mixture, with synergistic effects increasing towards the equator.

4. Synthesis. These results highlight the importance of soil fauna in mixed litter decomposition, most strongly in dry environments. In this era of global climate change, where some regions are projected to become drier, soil fauna might compensate the expected slower decomposition of litter by increasingly enhancing litter mixture decomposition.
1 | INTRODUCTION

Litter decomposition governs carbon and nutrient cycling in terrestrial ecosystems (Brovkin et al., 2012; Sitch et al., 2003). Over 50% of net primary production in terrestrial ecosystems returns to the soil through litter fall and decomposition (Cebrian, 1999). Climate, decomposers (including micro-organisms and soil fauna), plant litter diversity and litter quality together drive litter decomposition (Aerts, 1997; García-Palacios et al., 2013; Gonzalez & Seastedt, 2001; Parton et al., 2007).

Precipitation is a key climate factor that impacts litter decomposition processes in terrestrial ecosystems (Aerts, 1997; Taylor et al., 2017; Yu et al., 2019), by promoting leaching of water-soluble nutrients and phenolic compounds in the litter (Deng et al., 2018) and microbial activity through soil water availability (Beier et al., 2012). Changes in the soil water availability also affect the abundance and composition of the soil fauna (Andriuzzi et al., 2020; Song et al., 2016), thus exerting considerable influence on the litter decomposition rate (Handa et al., 2014; Hättenschwiler et al., 2005).

Recent research suggests that litter quality could surpass climate in controlling decomposition rates across biomes globally (Cornelissen et al., 2017; Cornwell et al., 2008; Hoeber et al., 2020). Litter quality, for example, nutrient content or carbon-based defence compounds, is often species specific (Cassart et al., 2020; Cornwell et al., 2008; Güsewell, 2004) and as a result, litter decomposability tends to be species specific. Furthermore, litter decomposability can vary with litter species richness when in mixture (Gartner & Cardon, 2004; Leppert et al., 2017). In natural ecosystems, plants co-occur in mixtures; therefore, litter mixtures are of different qualities in different ecosystems (Grossman et al., 2020; Handa et al., 2014; Jiang et al., 2019; Porre et al., 2020). Initial carbon (C), nitrogen (N), lignin and phosphorus (P) concentrations have been shown to influence decomposition and their dissimilarity in litter mixtures could influence the decomposition of litter mixture (Cassart et al., 2020; Gao et al., 2016; Vos et al., 2013). When a mixture is composed of chemically dissimilar litters, microbial transfer and leaching of nutrients from litters with high concentrations to those with lower nutrients concentration may occur (Gessner et al., 2010). Such dissimilarity in litter mixtures is likely to boost litter mixture effects. Nevertheless, a few studies did not find such results. For example, Hoorens et al. (2003) reported that initial litter dissimilarities explained none of the litter mixture effects in their experiment. According to these authors, it is difficult to characterize the quality of litter based on the single litter chemical traits. This may partly be because, within litter mixtures, possible interactions resulting from one litter chemical trait (e.g. N) could be modulated by other litter chemical traits (e.g. lignin or phenolics). Furthermore, the diversity of litter in mixtures could also promote fauna abundance and diversity by creating a favourable microclimatic condition in the litter layer and therefore could accelerate litter decomposition (Gao et al., 2016). Thus, flora biological diversity, whether at species or genotype levels, likely impacts significantly litter decomposition and thus nutrient cycling in forest ecosystems (Hättenschwiler, 2005).

Both climate and litter quality may directly or indirectly determine litter decomposition rates and the magnitude of their effects could be modulated by the presence of decomposers (Fujii et al., 2018). Decomposers play a central role in litter decomposition and studies generally show that they accelerate the decomposition process (Schädler & Brandl, 2005; Wang et al., 2018; Yang & Chen, 2009). Their individual contributions to decomposition vary from physical breakdown to ‘biological’ mineralization of litter from different plant species where decomposers breakdown the litter into forms that are readily available to plants (Hättenschwiler & Gasser, 2005; Hättenschwiler et al., 2005). Micro-organisms are predominantly responsible for chemical transformation, while invertebrates have a predominant role in physical breakdown of the litter (but see Griffiths et al., 2021 for a review of their direct role in chemical transformation). Here, we focus on invertebrate soil fauna, which, besides a non-negligible direct role in chemical breakdown (Griffiths et al., 2021), affect litter decomposition via three mechanisms: (i) physical breakdown to smaller pieces, (ii) consumption of small pieces and (iii) exposing litter to micro-organisms’ colonization; and together these mechanisms will result in mass loss (David & Handa, 2010; Hättenschwiler et al., 2005). The indirect influence of fauna on litter decomposition also exists at multiple trophic levels (Gessner et al., 2010). The interactions between litter and soil fauna affect the process of litter decomposition either positively (Handa et al., 2014; Vos et al., 2013) or negatively via preferential feeding (Swan & Palmer, 2006b).

Plant litter diversity in mixtures can affect litter decomposition in two ways: (i) through disparities in litter species composition, leading to transfer of nutrients among species and (ii) by changing the microenvironment for litter decomposition ( Hector et al., 2000). Changes of litter quality dissimilarity in litter mixtures influence the mixtures decomposition directly or indirectly through exchange of complementary resources among decomposers (Cassart et al., 2020; Guo et al., 2019; Vos et al., 2013). Differences in species composition in litter mixture can influence the feeding behaviours of soil fauna and their abundance; hence, they influence leaf litter decomposition (Santonja et al., 2017). Several studies have investigated the decomposition of litter mixtures and ambiguity prevails with regards to the outcome of litter mixture decomposition. Previous studies have brought forth different mechanistic hypotheses as possible explanations for the effects of diversity on decomposition. Here we only consider three generalized competing hypotheses: (i) The ‘additive effects hypothesis’ which stipulates that litter decomposition is irresponsive to changes in plant diversity hence additive effects

KEYWORDS
biodiversity, drought, ecosystems functions, global analysis, litter decomposition, litter mixture effect, nutrient cycling, soil fauna
individual species and interactions among species are complex and diversity changes, ecosystem functions also change, but in a non-
2018). And (iii) the ‘idiosyncratic hypothesis’ stipulating that when diversity changes, ecosystechnic functions also change, but in a non-
and variable (Lawton, 1994). Therefore, in light of the existing mechanistic hypotheses from empirical studies on the decomposition of litter mixtures, it remains impossible to determine the direction of litter mixture decomposition prior to conducting an experiment. Litter diversity accelerates or decelerates, and sometimes has no effect on litter decomposition (but see Mori et al., 2020).

One reason behind these contradicting outcomes of litter diversity experiments could be the fact that many studies fail to account for the effect of soil fauna (Patoine et al., 2017; Peguero et al., 2019). The exclusion of soil fauna by use of fine mesh size litterbags in some studies and inclusion of soil fauna by use of coarse mesh size litterbags in others have led to the observed discrepancies in the outcome of litter mixtures decomposition experiments. The interactions between soil fauna and litter diversity are poorly understood (Liu et al., 2019) and experiments evaluating litter diversity and soil fauna interactions are rare (but see Zhou et al., 2020). This calls for a full consideration of both litter diversity and soil fauna, which would lead to a holistic understanding of the outcome of decomposition of litter mixtures. Litter mixtures and soil fauna are likely to interactively affect litter mixture decomposition. Litter species diversity affects the quality, which, in turn, affects soil fauna feeding behaviour as they are sensitive to litter quality changes (Hassall et al., 1987; Yang & Chen, 2009). Differences in litter traits in mixtures could favour high abundance and diversity of soil fauna and hence the soil fauna influence on litter decomposition could respond to the litter mixture diversity (Hättenschwiler & Gasser, 2005; Hättenschwiler et al., 2005). Thereto, we carried out a global meta-analysis as a powerful tool to derive conclusions from multiple single studies with their environmental and methodological idiosyncrasies (Gurevitch & Hedges, 1999).

Furthermore, the interaction between soil fauna and litter diversity could be affected by other factors like climatic conditions. For example, the levels of precipitation could either reduce or amplify the effect size of soil fauna and litter mixture on decomposition. Here, we investigated how variation in precipitation influences these interactions. We attempted to answer the following overarching questions: (1) Does the presence of soil fauna change the litter mixture effect? And (2) If soil fauna changes the litter mixture effect, is that impact influenced by precipitation levels? To answer these questions, we first have to consider the overall mixture effect without taking into account faunal effects.

We hypothesized that (1) litter mixtures will decompose faster than expected based on the component species singly. Based on previous literature (Mori et al., 2020; Srivastava et al., 2009), this effect size will saturate with an increase in litter species richness beyond two species in mixture. This is because, on the one hand, increased species richness will provide diverse food resources that support a wider range of micro-organisms and soil fauna, hence increasing decomposition (complementary resource utilization). On the other hand, this positive effect should saturate when overlap in resource quality among species becomes stronger. (2) Including the effects of soil fauna in litter mixture experiments will cause a positive change in the magnitude of the mixture effect on litter decomposition. The presence of soil fauna will lead to accelerated rates of decomposition for the litter mixture while the absence of soil fauna will lead to decelerated rates of decomposition compared to the expected decomposition rate from the component single species. This is because soil fauna could directly (e.g. consumption) or indirectly (e.g. physical breakdown leading to greater micro-organisms access) enhance litter decomposition (Hättenschwiler & Gasser, 2005; Hättenschwiler et al., 2005). (3) With lower precipitation, soil fauna will have more positive impact on the litter mixture effect. In environments with low precipitation, nutrient loss in the litter layer due to leaching will be reduced; therefore, soil fauna consumption will be relatively higher. Litters that are more physically diverse (leaf shape, sizes and surface structure) create a more stable microclimate and hence a better micro-habitat for the fauna (Hättenschwiler et al., 2005; Makkonen et al., 2013). For instance, litter mixtures could also increase litter layer moisture retention by filling the available internal volume more (Makkonen et al., 2013; Wardle et al., 2003) and hence favour fauna activities resulting in increased rates of decomposition. By contrast, at higher precipitation levels, there will be periods of poor oxygen availability (hypoxia) resulting from flooding or sediments deposition on the litter layer that could disrupt normal fauna functioning and hence decreased the fauna effect (Tillman et al., 2003). Moreover, runoff could also disrupt the abundance and diversity of soil fauna, hence reducing soil fauna effect on decomposition of litter mixtures. These hypotheses are visualized in a conceptual framework (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Data compilation

We performed a literature search in the ISI Web of Knowledge (http://isiknowledge.com) on 30th May 2020. The searching terms were (leaf litter* or leaf debr* or leaf residue* mixing or litter biodiversity or litter drivers* or non-additive effects or additive effects) AND (litter decompos* or decay* or degrad*) AND (litterbags). The search resulted in 950 publications. Initial assessment of study relevance was made on the title and abstract of each paper and this trimmed the total number of potential papers to 174 publications. We then examined the full text of the identified papers and extracted data from those that fulfilled the following inclusion criteria: (1) Field studies of litter decomposition experiment that used litterbags with given mesh sizes to explicitly exclude soil fauna, (2) mass loss was reported for both single-species litters and multiple-species litters, and (3) the biomass ratio of the litters in the mixture was reported.
Out of the 174 papers, 40 met our inclusion criteria. A literature search using the same search terms was again performed on 30th June on the Scopus database, which yielded 328 papers. Out of the 328 additional papers, we got 70 new papers that were not in the ISI Web of Knowledge search results. Among them, 15 papers met the inclusion criteria. This added up to 55 papers in total from which data were extracted. A list of the papers is provided at the data source section. In total, 873 observations were recorded across 60 different sites in 20 countries (Figure 2) out of the 873 observations only 274 observations considered soil fauna. When the same litter combination was used at different sites or different mesh sizes, they were considered as separate observations. Different litter combinations at the same site and time were also counted as different observations. Where the experiments involved any kind of manipulation (e.g. addition of nutrients, precipitation exclusion or inclusion), we only extracted data for the control plots (i.e. free from any manipulation).

We extracted the mean of the mass loss variable (see below), its standard deviation and the litterbag number of replicates (sample size n) on which these results were based, from tables, figures, main text and from supplementary data from the studies identified. We used the 9.8 version of Engauge digitizer software to extract data from figures when the data were not given in text or Supporting Information. Where the standard deviation of the mean was not explicitly given, we extracted the standard errors, and the P values comparing the actual litter mixture's mass loss (observed mass loss) and the predicted litter mixture mass loss based on single-species litter (expected mass loss). The observed mass loss was always based on a litter mixture and the expected mass loss on the mean mass loss values of the respective single species that comprised the mixture. We then used the Revman calculator to estimate the standard deviation from the standard errors and P values (https://training.cochrane.org/resource/revman-calculator). We requested data directly from corresponding authors for publications that did not report or provide data that could be used to calculate the standard deviation. For the expected mass loss standard deviation, when not given, we calculated the pooled standard deviation (weighted average of standard deviations for more than one group) (Cohen, 1988) following Equation 1.

$$S_{\text{Pooled}} = \sqrt{\frac{S_1^2 + S_2^2 + \cdots + S_k^2}{k}},$$

where $S_1$, $S_2$ and $S_k$ are the standard deviation of mean mass loss of the component species and $k$ is the number of species. We also collected

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**FIGURE 1** Hypothetical framework of this study. Litter mixture species richness and soil fauna are experimentally manipulated to depict their respective effect on litter decomposition (Hypotheses 1 and 2) and how precipitation levels influence those interactions. H1—Multiple species litter will have higher decomposition rate than the component single-species litter in the mixture but this effect will soon saturate with an increase in litter species richness in mixture. H2—Inclusion of soil fauna will cause a change in the magnitude and direction of litter decomposition of multiple-species experiments, but not in single-species experiments. H3—With decreased precipitation, soil fauna will have a greater positive impact on the decomposition of litter mixtures, although we acknowledge a potential negative effect if dominant soil faunal taxa exert a net preferential feeding behaviour (Swan & Palmer, 2006b)
data on the study sites’ geographical coordinates, litter-bag mesh size, annual precipitation and litter chemical traits to examine how these variables affected the litter mixture effect. When precipitation was not given in the source paper, we used WorldClim to get the approximate precipitation level of the experimental site using the raster package in R (Hijmans et al., 2012).

The observed and expected mass loss percentages for the litter mixture (see above) were extracted from the source papers. When the expected mass loss was not available, it was calculated as the mean mass loss of the component species following Equation 2:

\[
\text{Expected mass loss } \% = \sum f_i M_i. \tag{2}
\]

where \(M_i\) is the mass loss in % of a single species, and \(f_i\) is the mass fraction of each litter in the mixture. In cases where mass loss was not given, we calculated it from the given decomposition rate constant \((k)\) in the equations used to calculate \(k\) in the specific paper. When not given, the litter chemical traits for the mixtures were calculated as the average chemical content for the component species in the mixtures.

Using the litterbag mesh size given in the papers, we classified the data into two categories based on whether macrofauna and mesofauna had access to the litter mixture: fauna present (mesh size >1 mm) and fauna (except microfauna) absent (mesh size <1 mm). We also classified the study sites according to climate zone (tropical, subtropical and temperate regions) based on their latitude coordinates given in the paper. We considered precipitation as continuous variable to apprehend its effects on decomposition rates and we also classified annual precipitation into three groups (High: >2000 mm/year, Medium: 1000–2000 mm/year and Low: <1000 mm/year). Owing to the importance of time in litter decomposition experiments, we also evaluated how the incubation duration influences the outcome of litter mixture effect. Finally, we categorized the sites into shrubland, grassland, plantations and forests based on the ecosystem type in which litterbags were incubated. When not clearly indicated, we used the reported vegetation cover to classify them into different ecosystems.

2.2 | Statistical analysis

We used log response ratio (\(\ln (RR); \) Equation 3) to evaluate mass loss difference between species decomposing in mixtures and in single-species litters. The \(\ln (RR)\) has an advantage of small sampling distribution bias and it follows a nearly normal sampling distribution when the denominator standardized mean is large (Hedges et al., 1999). Also, the \(\ln (RR)\) takes into account the different durations of mass loss experiments. All studies had single species and litter mixtures composed of different number of species ranging from 2 to 12 species. To be consistent across studies, we used the expected mass loss as the control for the meta-analysis and the observed mass loss as the treatment:

\[
\ln (RR) = \ln \left( \frac{\text{observed mass loss } \%}{\text{expected mass loss } \%} \right). \tag{3}
\]

A positive value of \(\ln (RR)\) indicates that species mixtures enhance litter decomposition while a negative value indicates that species mixtures diminish litter decomposition. The variance for the \(\ln (RR)\) was calculated using Equation 4:

\[
V(\ln (RR)) = \frac{\left( S_e \right)^2}{N_e (S_e)^2} + \frac{\left( S_c \right)^2}{N_c (S_c)^2}, \tag{4}
\]

where \(N_e\) and \(N_c\) represent the sample sizes for the observed mass loss and the expected mass loss, respectively; \(S_e\) and \(S_c\) are the standard deviations for the observed mass loss and the expected mass loss, respectively.

We applied random-effects meta-analysis to avoid pseudo-replications and to account for independence in the data. The random-effects model uses the inverse of within-studies and
between-studies variance to weight the true effect sizes, and it accounts for autocorrelations in observations within each study (Zhang et al., 2018). The expected and the observed mass loss difference was considered significant if the 95% confidence interval did not overlap with zero.

We conducted a meta-regression using the metafor package to examine how the litter mixture effect changed across climatic regions, ecosystems, precipitation levels and how the presence of fauna influenced the litter mixture effect under these categories. We also performed a meta-regression using a mixed-effect model to test how species richness, incubation period and dissimilarity in litter chemical traits influenced the estimated effect size of litter mixture effect on the total observations. We used the QM (tests the amount of heterogeneity explained by the moderators) and their related p values to estimate how the moderators influenced the effect size (In (RR)). A correlation between the moderators was tested using Pearson correlation to determine the set of moderators to be included in the model. For variables that were highly correlated, we only included one of the variables in our full model. To find a model that best explains the effect size observed, we fit all possible models to our data and ranked the models based on the Akaike information criterion (AIC) using the glmmulti package (Calzado & de Mazancourt, 2010). Further analysis was restricted to the models within a difference of 2 AIC to the model with the lowest AIC. A total of 150 mixed models were fitted to our data (466 observations) since all observations with missing data for the moderators were left out from the analysis (see Table 1 for best model summary). All statistical analyses were conducted using the metafor package (Viechtbauer, 2010) in R software version 4.0.2 (R Core Team, 2020).

We visually checked the publication bias using a funnel plot between standard error and effect size and we observed that the funnel plot was symmetrical meaning that there was no publication bias (Figure S1). We also performed the Rosenberg fail-safe number analysis (Rosenberg, 2005), which gives an estimate of the potential non-significant studies that need to be added to change our conclusion. We can have confidence in our results because the fail-safe number was 155,317,469,437 which is greater than the recommended threshold 5k + 10 where k is our total number of observations (Rosenthal, 1979).

### 3 | RESULTS

#### 3.1 | Decomposition of litter mixtures

Litter mixtures effect sizes indicated that at times litter mixtures decompose slower or faster than the expected mass loss based on single-species litter. The overall effect size was however not significantly different from zero (additive effect; −0.006 ± 0.006, p = 0.346; see Figure 3), suggesting that litter decomposition does not generally deviate much from what would be expected from their constituent single-species litter. Meta-regression results indicated that mixture species richness in the total observations (including and excluding soil fauna) did not have a significant effect on the overall litter mixture effect size (Q_M = 0.933, p = 0.334, R² = 0.000; see also Figure S2).

#### 3.2 | Effect of soil fauna on litter mixture effect

When the observations were classified based on the availability of soil fauna, the effect sizes were significantly different from zero (non-additive effect) but with different directions (positive and negative effects). The subsets excluding fauna showed a decelerated decomposition (−0.023 ± 0.005, p < 0.001, Figure 3) while those allowing meso-fauna and macro-fauna presented an enhanced decomposition rate for the litter mixture (0.038 ± 0.014, p = 0.008, Figure 3). Soil fauna availability was also the second best individual moderator that explained the litter mixture decomposition effect on the overall data after climatic region using AIC (Table S1).

#### 3.3 | Precipitation, climatic region and ecosystem type

Annual precipitation did not have a significant effect on the litter mixture effect when considered as a continuous variable and when categorized into different precipitation levels. However, the influence of soil fauna on the effect size varied greatly with the precipitation and was stronger at low precipitation levels (Figure 4). When precipitation was considered as a continuous variable, we did not

<table>
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<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>Z value</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
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<td>0.0895</td>
<td>8.7218</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>Litter C</td>
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<tr>
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<td>0.0031</td>
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<tr>
<td>Tropical</td>
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<td>0.0352</td>
<td>−3.9644</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Table 1.** Results of the best model explaining variations in the litter mixing effects in the meta-analysis. The model was selected based on the AIC after fitting 150 mixed models to our data.
observe a linear relationship with soil fauna effect (Figure S3). When categorized by climatic region, the litter mixture effect was negative in the temperate region (\( Q_M = 8.700, p = 0.003, R^2 = 0.069 \)) and tropical (\( Q_M = 0.0002, p = 0.988, R^2 = 0.000 \)) regions. When categorized by ecosystems, the litter mixture effect was negative in plantations, additive in forest and shrubland and positive in grassland when the data were categorized into different ecosystems (Figure S2c).

### 3.4 Time and litter quality effect on litter mixture effect

The experimental duration showed a significant but weak effect on the overall effect size of mixture on mass loss (\( Q_M = 3.941, p = 0.047, R^2 = 0.007 \)). When the experimental duration was categorized (<6, 6–12, 12–24 and >24 months), a negative effect size was observed for the period between 6 months and 24 months. Below 6 months and above 24 months, the litter mixture effect size did not deviate from zero (additive effect; Figure S4). Initial litter carbon (\( Q_M = 60.073, p < 0.001, R^2 = 0.145 \)), lignin (\( Q_M = 4.0, p = 0.045, R^2 = 0.011 \)) and phosphorus (\( Q_M = 4.425, p = 0.035, R^2 = 0.010 \)) concentration had a significant but weak positive effect on the \( \ln(RR) \). Initial nitrogen, C:N and lignin:N was not related to the \( \ln(RR) \) (Table S1).

### 4 DISCUSSION

We find that, in our dataset including litterbag studies with different mesh sizes, litter mixture does not enhance litter decomposition globally. Underlying this overall pattern, plant species mixing enhances litter decomposition when soil fauna is present, whereas plant species mixing reduces decomposition in the absence of soil fauna. The positive effect of soil fauna on the litter mixture effect was greater under lower precipitation. We also observed that the
litter mixture effect was significantly positive in the absence of soil fauna at high precipitation level.

4.1 | Overall litter mixture effect

Contrary to our first hypothesis, the overall litter mixture effect on litter decomposition rate at global scale was not significantly different from what would be expected from the sum of the constituent single-species litters in the mixture. This result not only confirms previous results from Porre et al. (2020) and Srivastava et al. (2009) but also expands to a wider range of litter species richness in the mixtures (up to 12 species in our dataset) compared with mainly two species in Porre et al. (2020). However, it contrasts previous results that did find an overall positive effect of litter mixing on decomposition rates (Gartner & Cardon, 2004; Kou et al., 2020; Mori et al., 2020; Xiao et al., 2020). This contrast could result from the kind of analysis done in the different studies (see Table S2). For example, the Gartner and Cardon’s (2004) study was mainly a vote counting analysis which showed that 67% of litter mixtures decomposed in a non-additive manner. The difference could also arise from the inclusion of other ecosystems apart from terrestrial ecosystems (Mori et al., 2020) or a terrestrial focus on a single type of ecosystems like forests (Kou et al., 2020) in these studies. In addition, the discrepancies in patterns between these previous studies and ours might be related to some methodological differences, for example the selection of different mesh sizes by different previous authors. In some previous meta-analyses (Kou et al., 2020), when different mesh sizes had been used in individual studies, they only included one type, for example, when publications had reported 1-, 2- and 5-mm mesh size they only chose one mesh size, mainly 2 mm. However, in our study, we included all given mesh sizes in the individual studies.

The number of species in the mixture did not show any significant effect on the litter mixture effect size (H2). This confirms reports that the characteristic and composition of species in the litter mixture determine the litter mixture effect more than the number of species in the mixture per se (Lin & Zeng, 2018; Mori et al., 2020; Srivastava et al., 2009). This observation contradicts the ‘positive biodiversity–function hypothesis’ (e.g. Gessner et al., 2010). Further investigation about the ‘positive biodiversity–function hypothesis’ (using litter decomposition as the function) should emphasize species identity in the mixture, thereby putting focus on species with known special chemical or physical quality, which are more likely to affect other species (e.g. Sphagnum or other mosses, see Hoorens et al., 2010; Wardle et al., 2003).

4.2 | Soil fauna effect

Dissecting the potential effect of soil fauna on the outcome of litter mixture effects represents one of the main points of focus of our study. Indeed, the presence of soil fauna in decomposing litter had a strong and significant effect on the litter mixture effect. This confirmed our second hypothesis, which stated that inclusion of soil fauna in litter mixture decomposition would change both the direction and the magnitude of litter decomposition. We observed that litter mixing decelerated litter mass loss when litter mixture treatments excluded soil fauna but accelerated litter mass loss otherwise. Thus, soil fauna has a great influence on litter mixture decomposition (Peguero et al., 2019). The influence of fauna could be direct by feeding on the litter and facilitating litter fragmentation (García-Palacios et al., 2013). Besides, soil fauna also modify the microbial community structure by stimulating microbial growth and colonization through increasing the accessibility of the litter to fungi and bacteria (David, 2014; Hättenschwiler et al., 2005). Litter mixtures with species that are highly dissimilar (physically or chemically) are more likely to attract diverse fauna groups that enhance decomposition of the mixtures compared to single-species litter, for example, mixtures of vascular plants with Sphagnum mosses (Hoorens et al., 2010). This could explain the positive litter mixture effect observed in the presence of soil fauna. This result corroborates findings reported in both a meta-analysis on the non-additive effect in litter mixtures (Liu et al., 2020) and a field experiment in two tropical forests by Peguero et al. (2019). These latter authors noticed a 22.6% increase in litter decomposition when soil fauna was included in the decomposition of litter mixtures. Together, these findings suggest that soil fauna enhances litter decomposition in the mixture and plays a major role in driving positive litter mixture effects (Barantal et al., 2014; García-Palacios et al., 2013), evident at regional and global scales (Tan et al., 2020). The overall neutral effect for the total observations seems to have resulted from the co-occurrence of both positive and negative (non-additive) effects of litter mixture on decomposition, which perhaps cancelled out each other (Chen et al., 2019). In contrast to our positive effects, a recent meta-analysis (Porre et al., 2020) surprisingly reported that the inclusion of fauna did not have any overall effect on litter mixture effects. The observed differences might be driven by the number of species in the litter mixtures (mostly two in theirs), which limits the diversity of available food sources for different decomposers. Additionally, the fauna classification based on the litterbags mesh size adopted in their methodology (<0.01 and >0.01 mm to <2.0 and >2.0 mm) is different from ours (<1.0 and >1.0 mm for the absence and presence of fauna, respectively).

4.3 | Precipitation effects and soil fauna interference

Our findings revealed that the litter mixture effect did not change with precipitation overall. But the soil fauna contribution to the litter mixture effect was stronger at lower precipitation levels, in line with our third hypothesis. Precipitation directly enhances the moisture content of litter, which drives litter decomposition in terrestrial ecosystems and its influence could mask the effects of fauna when moisture is not a limiting factor (Keiser & Bradford, 2017). At high
precipitation levels, large portions of water-soluble materials in the litter are leached (Schreeg et al., 2013). The varying leachate could enhance carbon use efficiency of microorganisms and hence boost decomposition (Cotrufo et al., 2013, 2015). This could explain the observed positive litter mixture effect at high precipitation levels. On the other hand, increased leaching (of e.g. phenolic compounds that might inhibit decomposition) could also reduce the effects of soil fauna on litter decomposition. During leaching, the transfer of inhibitory compounds among litters in the mixture reduces soil fauna consumption. Additionally, leaching of nutrients from the litter occurs and it makes the litter less palatable to soil fauna (Srivastava et al., 2009). Together, these processes could explain the lower soil fauna contribution to litter mixture effect at high precipitation levels. This result, however, contrasts a recent study by Tan et al. (2020), which reported an increased fauna effect with increased moisture along an altitudinal gradient that increased soil fauna diversity. High precipitation could affect the soil fauna negatively, as excessive water levels may depress the activity of litter decomposers (Schuur, 2001) or strong leaching via excessive water could diminish available nutrients in litter mixtures, which then would not attract soil fauna colonization and thus reduce their foraging. High precipitation could also increase the transfer of inhibitory phenolic compounds that decreases the fauna effect in litter mixtures (Gessner et al., 2010). Furthermore, when low-quality litter species decompose singly or when nutrients have been leached out, soil fauna may need to consume more of the litter to compensate for the missing nutrients (compensatory feeding). This compensatory feeding may result in higher expected than observed mass loss in the mixture (Swan & Palmer, 2006a). When in mixtures however, soil fauna is presented with a variety of nutrients and they may therefore selectively consume much of a specific leaf litter with high quality instead (e.g. preferential feeding; Swan & Palmer, 2006a, 2006b). This preferential feeding by soil fauna on leaf litter species mixtures has been already reported to reduce litter mixture effects in aquatic environments (Swan & Palmer, 2006b). From this observation, we suggest that field studies combining litter mixture, soil fauna effect and climatic factors manipulations be conducted to further unravel how all these factors interact in shaping the decomposition process globally (Griffiths et al., 2021). To open this black box, such studies should preferably also sample the invertebrate decomposer communities inside the litter bags.

4.4 | Climatic region

The litter mixture effect on decomposition changed from negative to positive and increased with the decrease of latitude. Litter mixture effect size was negative in the temperate regions, changed to zero in the sub-tropics, then to positive in the tropics. These results might stem from the low soil fauna abundance at high latitudes compared to low latitudes, as invertebrate species diversity diminishes as the latitude increases towards the poles (Bardgett & Van Der Putten, 2014; Haanes & Gulliksen, 2011; Hawkins, 2001). Moreover, a decrease in temperature with an increase in latitude represents a possible driver of this trend, since soil fauna abundance and activities are limited by temperature (but see for nematodes van den Hoogen et al., 2019). Decreased temperature and reduced precipitation in a region might also retard fungal activity. This eventually affects the rate of decomposition and the litter mixture effect. The non-additive effect is also temperature sensitive, with non-additive occurrences increasing with increase in temperature (Duan et al., 2013). However, from our data, it is crucial to note that over 55% of the observations were from the temperate climate region. Therefore, in the future, more studies from lower latitude regions could improve the robustness of global analysis of litter mixture effects.

5 | Conclusions

This global synthesis reveals that, while litter mixtures on average decompose as fast as predicted from the average decomposition rates of their component single-species litters, the presence of soil fauna in litter mixture decomposition changes both the magnitude and direction of litter decomposition. Inclusion of soil fauna enhances decomposition of litter mixtures while exclusion of soil fauna diminishes decomposition of litter mixtures. Furthermore, we observed that the soil fauna effect in litter mixtures increases at reduced precipitation levels. We also observed that at high precipitation levels (>2000 mm/year), the litter mixture effect was positive in the absence of soil fauna but non-significant in the presence of fauna. Our results highlight the importance of soil fauna in promoting decomposition of litter mixtures and that future experiments need to consider soil fauna effects for a holistic understanding of litter mixture decomposition (see also Griffiths et al., 2021). Moreover, in this era of climate change, where some regions have been projected to experience drier conditions (IPCC, 2007), the increased fauna effect in these regions may partly compensate the expected lower rates of decomposition through an enhanced litter mixture effect. Therefore, the influence of climatic factors such as precipitation on the interactions between soil fauna and litter species in mixtures during decomposition should be investigated in more depth.

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CONFLICT OF INTEREST
All authors declare no conflict of interest.

AUTHORS’ CONTRIBUTIONS
All authors conceived the ideas and designed the methodology; D.M.N. collected the data; D.M.N., G.G.O.D. and S.-C.C. jointly analysed the data; D.M.N., G.G.O.D., S.-C.C. and J.Z. led the writing of the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

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