Functional identity drives tree species richness-induced increases in litterfall production and forest floor mass in young tree communities

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Summary:

1. Forest floor accumulation is a key process that influences ecosystem carbon cycling. Despite evidence suggesting that tree diversity and soil carbon are positively correlated, most soil carbon studies typically omit the response of the forest floor carbon to tree diversity loss.

2. Here, we evaluated how tree species richness affects forest floor mass and how this effect is mediated by litterfall production and forest floor decay rate in a tree diversity experiment in a subtropical forest.

3. We observed that greater tree species richness leads to higher forest floor accumulation at the soil surface through increasing litterfall production – positively linked to functional trait identity (i.e., community-weighted mean functional trait) rather than functional diversity – and unchanged forest floor decay. Interestinglystructural equation modeling revealed that this lack of overall significant tree species richness effect on forest floor decay rate was due to two indirect and opposite effects cancelling each other out. Indeed, tree species richness increased forest floor decay rate through increasing litterfall production while decreasing forest floor decay rate by increasing litter species richness.

4. Our reports of greater organic matter accumulation in the forest floor in species-rich forests suggest that tree diversity may have long-term and important effect on ecosystem carbon cycling and services.

Keywords: Ecosystem functioning, forest biodiversity, functional trait, litter decomposition, primary productivity, selection effect hypothesis

Introduction

Forest floor organic horizons primarily form from a wide spectrum of decomposition products, influence soil formation, serve as habitat of a wide diversity of soil organisms, and affect plant germination and community structure (Buol et al., 2011). Importantly, forest floor organic horizons act as sponges that turn rainfall deluges into life-supporting soil moisture and buffer temperature variation and extremes in underlying mineral horizons (Fisher & Binkley, 2019). In forests, large amounts of carbon are stored in forest floor organic horizons. At a global scale, organic matter in forest floor ranges between 15 and 100 Mg ha^{-1} in boreal forests, between 7.5 and 12.5 Mg ha^{-1} in temperate broadleaved forests, and between 1 and 2.5 Mg ha^{-1} in tropical forests (Vogt et al., 1986). Forest floor organic matter is more sensitive to forest management and climate changes, such as harvesting, reforestation, and drought, than mineral soil horizons (Davidson & Janssens, 2006; Jandl et al., 2007). It is thus key to developing sustainable management strategies to increase forest floor carbon as fast as possible after human disturbance and environmental change (Mayer et al., 2020). Recent studies have shown that greater tree species richness has a positive effect on ecosystem functions such as forest productivity (Huang et al., 2018) and soil organic carbon storage (Chen et al., 2020; Xu et al., 2020). Yet in these latter studies, the effect of tree species richness on forest floor mass remains unexplored.

Mass accumulation on forest floors depends on litter detrital inputs and decay rates (Hobbie et al., 2006). Tree species richness may promote forest floor mass through increasing litterfall production which accounts for one-third of net primary productivity in forest ecosystems (Clark et al., 2001). Previous studies reported a positive effect of tree species richness on forest productivity (Liang et al., 2016; Schnabel et al., 2021; Urgoiti et al., 2022). Complementarity effect hypothesis is often used to explain the positive relationship between richness and productivity. This hypothesis proposes that species-rich communities use available and limiting resources more efficiently through niche differentiation and canopy packing and thereby lead to higher productivity (Cardinale et al., 2011; Morin et al., 2011; Jucker et al., 2015). Alternatively, the selection effect hypothesis proposes that tree species richness effects on productivity are driven by the functional traits of dominant species in a community (i.e., functional identity) (Roscher et al., 2012; Chiang et al., 2016). The increased probability of high-yielding or dominant species by increasing species richness enhances the ecosystem productivity (Loreau & Hector, 2001; Urgoiti et al., 2022). However, whether the litterfall production increases significantly with tree species richness remains inconclusive. While a subtropical tree diversity experiment with five levels of species richness (1, 2, 4, 8, and 16) showed that increasing tree species richness promotes annual litterfall production (Huang et al., 2018b), another tree diversity experiment found that the highest litterfall production was detected at intermediate diverse levels (the tree species richness levels were 1, 3, and 6) (Scherer-Lorenzen et al., 2007). These varied results highlight the complexity of tree species richness effects on litterfall production in forests.

Tree species richness may also promote forest floor decay rates due to the increased litterfall production and due to litter species richness which can increase resource complementarity and improved microenvironmental conditions for decomposers (e.g., soil temperature, moisture, and pH) (Hättenschwiler et al., 2005a; Joly et al., 2017). Increasing tree species richness could accelerate litter decomposition due to its positive effect on litterfall production and temporal litterfall asynchrony (Huang et al., 2017). Decomposition of older forest floor organic matter may be accelerated by increased and asynchronous fresh litterfall inputs. Because energy and nutrients released during decomposition stimulate microbes, which increase the production of extracellular enzymes that not only degrade more fresh plant materials, but also a portion of the older organic matter (Fontaine et al., 2004; Fontaine et al., 2007). Litter species richness, as a consequence of tree species richness, could accelerate litter decomposition by providing a higher physically and chemically diverse substrates and increasing abundance and diversity of decomposer community (Scherber et al., 2010; Otsing et al., 2018; Xiao et al., 2020). Furthermore, tree species richness is expected to increase litter decomposition by providing better microclimatic conditions, such as reducing soil evapotranspiration and retaining soil humidity through plot-specific canopy closure (Joly et al., 2017; Gottschall et al., 2019). For example, compared to monospecific litter layers, species-rich litter layer has higher water-holding capacity and better radiative-energy balance, which has indirect and positive consequences for decomposition (Makkonen et al., 2013).

In this study, we investigated the response of litterfall production, forest floor decay rate, and forest floor mass to increasing tree species richness in a subtropical tree diversity experiment. We hypothesised that: (H1) tree species richness increases forest floor mass through increasing litterfall production - this hypothesis is based on the current evidence from the positive tree diversity - forest productivity relationships (Huang et al., 2018a; Urgoiti et al., 2022) and litterfall production is a useful indicator of forest productivity; (H2) tree species richness decreases forest floor mass via its positive effect on forest floor decay rate - this hypothesis is based on current evidence that increasing litter species richness increases resource complementarity and improves microenvironmental conditions for decomposers, thereby accelerating forest floor decay rates (Joly et al., 2017; Lin et al., 2021), and that consequently, (H3) tree species richness has no significant impact on forest floor mass, as the aforementioned effects (litterfall production increases and forest floor decay accelerates with tree species richness) cancel each other out.

Materials and methods

Site description and experimental design

The present study was carried out in the Baisha Forest Farm in Southwest Fujian Province, China (25°07′N, 116°32′E). The regional climate has a mean annual temperature of 19.8 °C and rainfall of 1637 mm, between 1971 and 2020. Based on the USDA soil taxonomy, the soils are classified as Oxisol. The site was previously used as a Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) plantation, the major planted forests in South China. In March 2019, we established a tree diversity experiment after clearing-out and slash-burning the Chinese fir plantation (Huang et al., 2022; Wan et al., 2022). In this experiment, we selected 32 tree species according to their life-history (shade and drought tolerance, Table S1). In total, 299 plots of 12 × 12 m were arranged in a randomized factorial design, with species richness level ranging from 1, 4, 8, 16, to 32 species (Fig. S1 and Table S2). Each plot was planted with 256 one-year-old tree seedlings and spaced approximately 0.75 m apart to promote rapid canopy closure and reduce the chances of invasion of non-target woody species. In species mixtures plots, all tree species were planted in equal proportions and we systematically assigned 256 tree seedlings to allow for the maximum possible mixture of inter-specific and -trait information (Huang et al., 2022).

Litterfall production, forest floor mass, and forest floor decay rate

The litterfall was collected monthly from April 2021 to August 2022 for seventeen months. In April 2022, the trees were four years old and the canopy in more than 70% of plots had closed. The mean species-specific height of trees ranged between 0.91 m (*Quercus variabilis*) and 3.89 m (*Mytilaria laosensis*) (Table S2). To collect the litter, we used litter traps made from plastic black box (47 cm × 47 cm and 20 cm depth), lined with a 0.5 cm × 0.5 cm mesh fabric to reduce water retention. Because of the intra-plot space heterogeneity, a litter trap can only collect litterfall from the four adjacent trees. To ensure that the collected litterfall accurately represents the plot-level, five litter traps were placed in monocultures and 4-species mixtures, six were placed in 8-species mixtures, and ten were placed in 16- and 32-species mixtures (Fig. S2).

In the laboratory, the collected litter material was separated into leaves, branches, reproductive organs (flowers, fruit, and seeds), and miscellanea (caterpillar frass, dust, dead insects, and unidentifiable parts). All samples were oven dried at 60 °C and weighed. Total litterfall production (g m^{-2} $year^{-1}$) was calculated as the sum of the average monthly amount of the dried biomass of all litter material within the plot. The proportion of leaf litter in the total litterfall production ranged between 53% and 100%. To determine the individual contribution of each planted tree species to the litterfall in the mixed forests, all leaves and flowers were separated by the planted tree species in October 2021. Litter species richness was assessed as the number of species identified in litter traps.

Since litter of subtropical evergreen trees is mainly produced in spring or autumn (Zhang et al., 2014), we estimated the forest floor mass in November 2021 and August 2022, one month before and after major litterfall, respectively. Within each plot, the entire forest floor organic horizon, immediately before reaching the mineral soil, was collected using six 50 cm × 50 cm frames. Each frame was placed randomly in areas near the litterfall collectors. All the organic horizon materials were oven dried at 60 °C and weighed. The forest floor mass (g m^{-2}) was calculated as the average of the two sampling dates.

We also calculated the forest floor decay rates (k, $year^{-1}$) using Olson's equation for discrete autumn litterfall as follows (Hobbie et al., 2006):

$$k = -\ln\left(1 - \frac{L}{L+F}\right) \quad \text{(Eqn. 1)}$$

where L is the annual litterfall production (g m^{-2} $year^{-1}$) from April 2021 to March 2022, and F if the forest floor mass (g m^{-2}). During the study period, there were no existing organic horizons on the soil surface under the monoculture plots of two coniferous species (*Cunninghamia lanceolata*, *Taxus wallichiana*) and one broadleaved species (*Acer palmatum*) and the three species did not produce any litterfall during the study period. Therefore, these plots were excluded, leading to a total of 283 experimental plots.

Functional trait measurements

To determine the effects of host tree community on litterfall production, forest floor mass and decay rate, we measured four physical and chemical traits of living leaves including leaf thickness (mm), specific leaf area (SLA, m^2 kg^{-1}), and leaf dry matter content (LDMC, mg g^{-1}), and leaf nitrogen content (%). These traits represent a species' resource (including light, water, and nutrient) capture efficiency (Soudzilovskaia et al., 2013) and are closely related to biomass production and rates of litter decay (Cornwell et al., 2008; Liu et al., 2018). A total of twenty fully mature leaves from ten individuals were collected for each tree species. For each leaf, the leaf thickness was measured with a micrometer. Leaf area was determined by a desktop scanner (Epson Expression 10000XL scanner) at a resolution of 400 dpi. All leaves were oven-dried at 60°C for 48h and weighted. The specific leaf area was calculated by dividing the leaf area by its dry weight. The leaf dry matter content was calculated as the ratio of its oven-dried mass to its water-saturated fresh mass. We measured leaf nitrogen content (%) using an elemental analyzer (Elemental EL III, Germany).

In addition to the above traits, we measured the total non-structural carbohydrate, condensed tannins, and total phenolic contents, lignin, and cellulose content of newly senesced leaf litter. Total non-structural carbohydrate are the sum of soluble sugars and starch, which were extracted by a methanol: chloroform: water solution and quantified after acid hydrolysis with concentrated sulphuric acid and Anthrone ethyl acetate reagent. Condensed tannins were analysed colorimetrically after hydrolysis with methyl alcohol using the proanthocyanidin assay (Preston et al., 1997). Total phenolics were analyzed using the Folin-Ciocalteau's reagent technique of Singleton and Rossi (1965). The contents of lignin and cellulose were determined by preextraction of acid-detergent fibre (Rowland & Roberts, 1994).

Tree growth and leaf area index

Individual tree height and basal diameter (GD, mm) above 5 cm ground level were measured at the end of 2021. Ground basal areas for individual trees were calculated as $\pi \times (GD/2)^2$. The total ground basal area of each tree species in mixed plots was calculated by adding all its ground basal areas together. With a LAI-2000 plant canopy analyser (Li-COR, USA), leaf area index was measured at 1.5 m above ground level in the fields.

Soil properties

Soil samples were taken in August 2021. Forest floor materials were carefully removed before the soil cores were collected. We took ten soil cores (3.5 cm diameter) per plot up to a depth of 10 cm and pooled to make one composite sample. The sampling locations were randomly selected in the midway between tree rows. For the plots with 16 and 32 species, we recorded the tree species surrounding each soil core and adjusted the soil core location to make sure that soil cores were collected under all species in the plot. Soil samples were sieved to 2 mm and dried naturally for three days prior to processing. Dried soil samples were ground with a ball mill and total carbon and nitrogen contents were analyzed by using an elemental analyzer. The pH values of soil samples were measured (1:2.5 soil to solution) in water. Soil temperature and moisture at 10 cm depth were monthly determined in the field by using a time domain reflectometry (TDR) unit (Model TDR300, USA) with two 12 cm long rods vertically inserted into the ground.

Statistical analyses

We calculated the functional identity (CWM, community-weighted mean) and diversity (FD, functional dispersion) for each mixed tree species plot using the "*FD*" package in R (Laliberté & Legendre, 2010). To test the relationship between biomass productivity and host ree community functional characteristics, the FD and CWM indices of leaves in the mixed tree species plots were calculated on the basis of the relative proportion of ground basal area for each tree species per plot. To test the contribution of leaf litter composition and diversity on forest floor decay rate, the FD and CWM indices of leaf litters were calculated for mixed tree species plots based on the relative proportion of litterfall produced by each species.

First, to explore the effect of tree species richness on the litterfall production, forest floor decay rate, and forest floor mass, we conducted linear mixed-effects models with tree species richness (TSR) and abiotic soil properties (Soil, that is soil pH, C/N, temperature, and moisture) as fixed factor:

$$Y = \beta_0 + \beta_1 \times TSR + \beta_2 \times Soil + \pi_{species} + \varepsilon \qquad \text{(Eqn. 2)}$$

where Y is the response variables (litterfall production, forest floor decay rate, and forest floor mass); β_i represented coefficients; $\pi_{species}$ is the random effect factor of tree species composition, accounting for spatial autocorrelation between measurements on samplings within the same tree species composition (Chen et al., 2022); ε is sampling error. To prevent overfitting, we selected the most parsimonious model among all alternatives with the condition to retain tree species richness as it was part of our core hypotheses to be tested. The model selection was accomplished by using the '*dredge*' function of the "*MuMIn*" package (Barton, 2018).

Second, to assess how the tree species richness effect was mediated by functional trait diversity and identity, the following model was applied to examine the effects of tree species richness, FD, or CWM indices, and their interactions on response variables (Y):

$$Y = \beta_0 + \beta_1 \times TSR + \beta_2 \times FD\ (or\ CWM) + \beta_3 \times TSR \times FD\ (or\ CWM) + \beta_4 \times Soil + \pi_{species} + \varepsilon \qquad \text{(Eqn. 3)}$$

To prevent overfitting, we added one FD (or one CWM) predictor at a time into the model (Eqn. 3). All predictive variables were scaled with the "*scale*" function prior to analysis to ensure that beta parameter estimates were comparable (Cohen et al., 2014). The linear mixed-effects models are fitted using restricted maximum likelihood estimation with the "*lme4*" package (Bates et al., 2014). The "*glmm.hp*" package was used to compute the individual marginal R^2 (R^2m) of each fixed factor in linear mixed-effects models (Lai et al., 2022). The Shapiro-Wilk and Levene tests were used to check the assumptions of normality and homogeneous variance, respectively. We performed square-root transformations on forest floor mass, litterfall production, and forest floor decay rate to improve the normality of residuals.

Third, we performed structural equation modeling (SEM) to disentangle the pathways through which tree species richness influences litterfall production, forest floor decay rate, and forest floor mass. First, we proposed an *a priori* SEM model to evaluate the direct and indirect effects of tree species richness, FD or CWM of living leaf traits, and leaf area index on litterfall production (Fig. S3a). Second, we proposed an *a* *priori* SEM model to evaluate the direct and indirect effects of tree species richness, litter species richness, abiotic soil properties, litterfall production, and forest floor decay rates on forest floor mass (Fig. S3b). Then, the fit of the reduced model was assessed by goodness-of-fit statistics and we selected the final model with the lowest AIC value (Grace, 2006). The Fisher's C statistic was used to calculate the goodness of fit of the model, and Shipley's test of d-separation was used to test whether any paths were missing from the model (Shipley, 2000). Our structural equation models were implemented using the "*piecewiseSEM* 2.1.2" package (Lefcheck, 2016). Plots with more species are rarer, with only four plots under 32 species out of 299 plots, we evaluated the robustness of our results by running our analyses by excluding the four 32-species plots and both the 16- and 32-species plots. All statistical analyses were performed in R version 4.2.1 (R Core Team, 2022).

Results

The annual litterfall production at the plot-level during the study period ranged from 10.5 to 896.3 g m^{-2} yr^{-1} (Fig. 1a). The forest floor mass at the plot-level ranged between 5.5 to 620.5 g m^{-2} (Fig. 1b). The monthly total litterfall peaked in March, September and November (Fig. S4). The forest floor decay rate (the decomposition constant (k) ranged between 0.1 and 3.8 yr^{-1} across all the study plots.

Annual litterfall production significantly increased with tree species richness ($R^2m = 0.04$, $P = 0.023$, Fig. 2a and Table S3). However, the forest floor decay rate did not change significantly with tree species richness ($R^2m = 0.001$, $P = 0.775$, Fig. 2b and Table S3). Consequently, the forest floor mass marginally increased with tree species richness ($R^2m = 0.03$, $P = 0.085$, Fig. 2c and Table S3).

We found that annual litterfall production significantly decreased with functional diversities in leaf thickness ($R^2m = 0.02$, $P = 0.008$) and leaf N content ($R^2m = 0.01$, $P = 0.031$, Table 1 and Fig. 3). Annual litterfall production significantly increased with CWM of specific leaf area ($R^2m = 0.02$, $P = 0.010$, Fig. 3c). In addition, we found a significant interaction effect between tree species richness and FD of leaf thickness and leaf N, and CWM of specific leaf area (Table 1). On average, litterfall production increased with tree species richness, with a stronger positive effect in forests with a higher CWM of specific leaf area and lower FD of leaf thickness and leaf N (Fig. S5).

The functional diversity of living leaf and leaf litter traits had no significant effect on forest floor decay rates (Tables 1 and S4). We found that the CWM of leaf N content had a significant and positive effect on forest floor decay rate ($R^2m = 0.01$, $P = 0.047$, Table 1). Our structural equation model showed that on the one hand, tree species richness had a positive and indirect effect on forest floor decay rate via its positive effect on litterfall production (Fig. 4). On the other hand, tree species richness had a negative and indirect effect on forest floor decay rate via its positive effect on litter species richness which in turn had a negative effect on forest floor decay rate (Fig. 4). In addition, forest floor decay rate significantly increased with increasing litterfall production, and decreased with increasing litter species richness (Fig. 5).

Forest floor mass decreased significantly with CWM of leaf N content ($R^2m = 0.10$, $P = 0.008$) and leaf dry matter content ($R^2m = 0.03$, $P = 0.005$, Fig. 6 and Table 1). The structural equation model accounted for 86% of the variation in forest floor mass (Fig. 4) and suggested that tree species richness has a positive and indirect effect on forest floor mass via its positive effect on litterfall production. In addition, we noted that the estimates of fixed effects were consistently similar between models with or without the 32 species mixtures (Figs. S6-S10) or without both the 16 and 32 species mixtures (Figs. S11-S15).

Discussion

Positive effect of tree species richness on litterfall production and forest floor mass

In support of our first prediction, greater tree species richness led to higher litterfall production, which is in line with previous studies (Huang et al., 2018b; Zheng et al., 2019; Maxwell et al., 2022). The niche complementarity and selection effect hypotheses are two important mechanisms used to explain the positive relationship between tree diversity and canopy production (Loreau & Hector, 2001; Fargione et al., 2007). For example, in a natural forest, tree species richness increased litterfall production via tree crown complementarity (Zheng et al., 2019). However, our results revealed that the identity of functional traits, especially specific leaf area, significantly increased litterfall production (Fig. 3). Our results mean that functional traits allow generalising to the prediction of ecosystem processes such as canopy productivity.

The positive relationships between CWM of specific leaf area and litterfall production suggests that stands composed of tree species with a high value of specific leaf area promote litterfall production and forest floor accumulation, thus supporting the selection effect hypothesis (Tobner et al., 2016; Grossman et al., 2017). We also found that increasing tree species richness accompanied by an increase in CWM of specific leaf area significantly increased litterfall production (Fig. S5). In other words, tree species with higher litterfall production in monoculture benefited from reduced competition when growing in mixtures, dominating them and overyielding in litterfall. Species that significantly enhanced litterfall production in mixture compared to monocultures were those species with greater specific leaf area. This concurs with evidence that the higher CWM of specific leaf area leads to fast growth and higher biomass accumulation (Reich, 2014; Finegan et al., 2015). Higher CWM of specific leaf area also leads to greater light interception and photosynthetic potential, leading to grater carbon uptake at leaf and stand scales. Unexpectedly, our results showed that the CWM of specific leaf area had an indirect effect on litterfall production via its negative effect on leaf area index. A possible explanation for the negative correlation between CWM of specific leaf area and leaf area index is that tree species with high specific leaf area often have short leaf life span (Reich 1992). Because we reported the values of leaf area index based on the averages of four seasonal measurements, our measure of leaf area index may be positively correlated with leaf life span (Gower et al., 1993 and our unpublished data). It is therefore possible that CWM of specific leaf area had a negative and indirect impact on leaf area index, a key canopy trait that harvest light. However, our results suggested that overall, high CWM of specific leaf area – which indicates a community dominated by individuals of fast-growing acquisitive species – lead to higher litterfall production, and may therefore lead to more organic matter accumulation in the forest floor.

Functional diversity of leaf thickness and leaf nitrogen content had negative effects on annual litterfall production, which can be in part due to the relatively greater importance of selection effects. Previous study indicated that the effect of functional diversity on forest productivity can become larger than the average effect of CWM values only after seven years of forest development (Bongers et al., 2021). In addition, the stress gradient hypothesis states that positive complementarity effects are important in stressful environments (Bertnessnd Callaway (1994). For example, studies conducted in herbaceous communities reported that plant mixture significantly reduced competition and increased complementarity in low fertility soils and the complementary effect decreased with increased soil nutrient availabilities (Wacker et al., 2008; Jarchow & Liebman, 2012). In addition, more productive plant species become increasingly dominated in nutrient-rich soils and under this environmental condition the relationship between plant diversity and ecosystem functions shows stronger selection effect (Tobner et al., 2016). In our study, the slash and burning were used for site preparation before the establishment of the experimental plots. The resulting layer of ash provides the newly-reforested plots with nutrient-abundant conditions, possibly favouring single species effects. Furthermore, light competition may contribute to stronger selection effects at the early stage of forest development (Tobner et al., 2016). High light conditions due to the early forest establishment may favour fast-growing (e.g., high CWM of specific leaf area) and shade-intolerant tree species over slow-growing and shade-tolerant growth in mixtures. Given that more than 50% of natural and secondary forests consist of > 16 species in subtropical China (Chen et al., 2020), our results suggest that forests with high tree species richness, and dominated by individuals of fast-growing acquisitive species, would quickly increase their forest floor carbon after disturbance.

Tree species richness had no overall effect on forest floor decay rate

Previous studies reported that effects of tree species richness on litter decay rates ranging from positive (Jacob et al., 2009; Joly et al., 2017), negative (Seidelmann et al., 2016; Gottschall et al., 2022), and neutral (Scherer-Lorenzen et al., 2007; Jewell et al., 2017). However, most studies of the relationships between tree diversity and litter decay rate often used litter bag methods and disregarded the fact that litter composition in the litterbags were not matched with their host tree communities (e.g., plant composition), which could not measure the combined effect of the host tree community and the produced litter on litter decomposition (Jewell et al., 2017; Joly et al., 2023). Litterbags have significant limitations, including exclusion of larger soil animals from litterbags by using mesh that is smaller than their body width. Previous evidence showed that soil fauna access to the litterbag determines the degree and direction of the influence of litter diversity on litter decomposition (Hättenschwiler & Gasser, 2005b; Njoroge et al., 2022). Confounding factors associated with litterbags include microclimate, handling and leaching losses, and the proportion of fragmented, undecomposed litter that falls or moves out of litterbags in the field. In addition, most studies of these litter decay rate only measure leaf litter, with no consideration of the decomposition of non-leaf components (e.g., branches, fruits, or flowers). After accounting for these factors and including other components of litterfall, we found that tree species richness had no significant overall effect on forest floor decay rate, which is contrary to our second hypothesis. Our results support the evidence that tree species richness effects on forest floor decay rate are multicausal due to combined litterfall production and litter species richness effects (Beugnon, 2021).

Interestingly, our SEMs showed that the absence of overall tree species richness on forest floor decay rates was due to two respectively positive and negative indirect effects cancelling each out other. On the one hand, tree species richness enhanced litter decay rates indirectly through increasing litterfall production, in line with results from Kavvadias et al. (2001) and Beugnon (2021). Litterfall is a major pathway of nutrient input from trees to soil. Increased litterfall production in species-rich forests my provide a large amount of new carbon source for soil decomposers (i.e., soil microbes and fauna) (Ebeling et al., 2014; Lin et al., 2021), and then promote the decomposition processes. In addition, increasing tree species richness would increase tree litterfall asynchrony (Morin et al., 2014; Huang et al., 2017). Fresh litter addition may enhance decomposition processes by a priming effect on microbial community (Creamer et al., 2015). However, this mechanism had not yet been tested. Therefore, future studies are needed to explore how tree species richness influences decomposition processes through its positive effect on temporal stability of litterfall production.

One the other hand, we found that litter species richness, which directly reflects tree species richness, had a negative effect on forest floor decay rate. This result contrasts with findings that litter species richness can accelerate litter decay by providing a higher physically and chemically diverse substrates and increasing abundance and diversity of decomposer community (Scherber et al., 2010; Otsing et al., 2018; Xiao et al., 2020). However, our results are consistent with other studies, where increasing leaf litter richness led to slower rates of litter decay in forests (Madritch & Cardinale, 2007; Grossman et al., 2020). Two potential mechanisms may account for the negative effect of litter species richness on forest floor decay rate. First, increasing litter species richness may slow down the decomposition because a functionally diverse mixture might result in leaching of inhibitory compounds (e.g., condensed tannins) between species (Hättenschwiler et al., 2005a;

Madritch & Cardinale, 2007). In the present study, we found that the CWM of leaf litter condensed tannins content increased with litter species richness (Fig. S16). Second, our previous study showed that an increase in tree species richness decreased soil total microbial biomass partially due to species-rich forests allocate less belowground biomass than young monocultures (Wan et al., 2022). Lower microbial biomass and activity may lead to lower forest floor decay rate (Chapman & Newman, 2010). However, interpretation of these findings should be done with caution. Indeed, since the forest floor decay rates were calculated based on twice measurements of forest floor mass, the sampling accuracy may not be very high for estimating long-term dynamics of forest-floor litter decay rates. Therefore, more accurate estimates of forest floor decay rate are needed to draw firm conclusions about these relationships.

Limitations and future directions

Our results highlight that greater tree species richness leads to more forest floor mass accumulation at the early stage of forest development. Despite this, the results of this work have several limitations. First, the early growth stage of trees is a complicated period for assessing tree diversity effects. The relationships between tree species richness, functional characteristics and forest litterfall production may change with stand development (Bongers et al., 2021). Further studies are needed to explore the temporal dynamics of the relationship between tree species richness and forest litterfall production. Second, the decomposer community (e.g., microorganisms and soil fauna) may not have adapted to the litter quality of the mixtures yet, which may affect the relationships between litter species richness and forest floor decay rates. Tree species richness may influence forest floor decay rate through its effect on decomposer community composition and diversity (Hättenschwiler et al., 2005b; Lin et al., 2021). Therefore, analyzing the community composition and diversity of decomposer at later stages of this tree diversity experiment will likely be helpful in understanding the ecological links between forest floor carbon dynamics and tree species richness.

Conclusions

There are three potentially important implications of our study. First, our study provides the first local scale evidence that increasing tree species richness could significantly increase litterfall production and forest floor mass. This is especially important in young forest plantations because soils under these forests were often recently disturbed due to forest floor clearing before the establishment phase. The soils under these forests have thin organic layers and forests often produce relatively low litterfall at young forest stand ages. Greater organic matter accumulated in the forest floor in species-rich forests may provide more resources and habitats for microorganisms, enhance soil moisture and stabilizes soil temperature through insulation. In the absence of enough litter layer (input), the favorable climate in tropical area likely promotes rapid mineralization of the extant soil C pool. Second, greater tree species richness led to higher forest floor mass accumulation at the soil surface through increasing litterfall production. This was driven by the identity of leaf functional traits, rather than the stronger complementarity effect generally reported in previous studies. This finding highlight that the species identity of a mixture may be more important than its functional diversity in determining tree species richness effects on ecosystem functions at early stages of tree growth. Last, tree species richness effects on forest floor dynamics are likely complex, depending on both litter quantity, quality and their complementary effects on forest floor decay rates. Tree species richness has no oerall effect forest floor decay rate. It enhanced litterfall production, in turn increased forest floor decay rate. However, tree species richness increases litter species richness, which decreased forest floor decay rate. This result indicated that tree species richness effects on forest floor decay rate are multicausal due to combined biomass and diversity effects. Our findings provide a more complete understanding of tree diversity effects on litter decay dynamics at the forest stand level by looking at forest floor dynamics rather than simply litterbag mass losses.

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Author contributions

ZH designed the research. XW analyzed the data. XW, HJ, MZ, and YF conducted field work and contributed to experiments and analysis. XW wrote the paper with contribution of ZH and F-XJ. All authors have reviewed and agreed with the manuscript.

Competing interests

The authors declare no competing interests.

Data availability statement

The data supporting the results is available from the Figshare Repository: http://doi.org/10.6084/m9.figshare.22807025

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Tables and Figures

Table 1 The effects of tree species richness (TSR), functional trait diversity (FD), community-weighted mean (CWM), and their interactions, and abiotic soil properties on the litterfall production, forest floor decay rate, and forest floor mass (Eqn. 3 in Methods, n=283).

Source of variation	Litterfall production		Forest floor decay rate		Forest floor mass	
	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
TSR	**1.66**	**0.002**	-0.01	0.806	**1.00**	**0.022**
FD of leaf thickness	**-1.19**	**0.008**	-0.01	0.945	-0.61	0.086
TSR* FD of leaf thickness	**-1.21**	**0.030**	<0.01	0.994	-0.74	0.092
R^2m	0.13		0.04		0.07	
R^2c	0.74		0.57		0.75	
TSR	**2.14**	**<0.001**	0.01	0.597	0.96	0.064
FD of leaf nitrogen	**-0.98**	**0.031**	-0.02	0.399	-0.42	0.253
TSR* FD of leaf nitrogen	**-1.38**	**0.033**	-0.03	0.324	-0.14	0.783
R^2m	0.15		0.05		0.07	
R^2c	0.70		0.59		0.73	
TSR	**0.99**	**0.082**	0.01	0.677	0.40	0.399
FD of SLA	0.089	0.844	-0.02	0.324	0.25	0.477
TSR* FD of SLA	0.28	0.659	-0.02	0.394	0.752	0.122
R^2m	0.11		0.05		0.06	
R^2c	0.70		0.58		0.75	
TSR	**1.08**	**0.067**	<-0.01	0.872	0.75	0.124
FD of LDMC	0.12	0.840	<-0.01	0.779	-0.03	0.952
TSR* FD of LDMC	-0.29	0.717	0.01	0.682	-0.35	0.599
R^2m	0.11		0.04		0.06	
R^2c	0.70		0.57		0.74	
TSR	**1.16**	**0.021**	-0.01	0.801	0.72	0.081
CWM of leaf thickness	-0.55	0.291	-0.03	0.101	0.08	0.837
TSR* CWM of leaf thickness	-0.80	0.223	0.03	0.174	-0.77	0.136
R^2m	0.11		0.12		0.08	
R^2c	0.72		0.58		0.74	
TSR	**0.92**	**0.048**	0.005	0.707	0.47	0.197
CWM of leaf nitrogen	-0.87	0.099	**0.04**	**0.047**	**-1.10**	**0.008**
TSR* CWM of leaf nitrogen	0.64	0.361	-0.05	0.062	0.67	0.224
R^2m	0.18		0.19		0.20	
R^2c	0.70		0.56		0.73	
TSR	**1.17**	**0.015**	-0.002	0.866	0.71	0.079
CWM of SLA	**1.27**	**0.010**	0.04	0.076	0.41	0.300
TSR* CWM of SLA	**1.40**	**0.042**	-0.02	0.588	0.81	0.143
R^2m	0.12		0.09		0.06	

R^2c	0.71		0.58		0.74	
TSR	**0.98**	**0.046**	<-0.01	0.976	0.49	0.226
CWM of LDMC	-0.96	0.096	<0.01	0.332	**-1.30**	**0.005**
TSR* CWM of LDMC	-0.51	0.487	<0.01	0.394	-1.04	0.070
R^2m	0.13		0.05		0.10	
R^2c	0.70		0.58		0.74	

Text in bold indicates a significant effect ($P < 0.05$). SLA, specific leaf area; LDMC, leaf dry matter content. R^2_m and R^2_c represent the level of deviance of the variable explained by all paths from the fixed effects and both the fixed and random effects ("species"), respectively.

Fig. 1 The tree species richness-level means of litterfall production (a) and forest floor mass (b). Values for boxplots are medians, with 75% of observations in the boxes, and whiskers above and below the boxes indicating the 95th and 5th percentiles.

Fig. 2 The effects of tree species richness on the litterfall production, forest floor decay rate, and forest floor mass. Blue lines and grey shaded areas represent fitted regressions and their bootstrapped 95% confidence intervals. The significance value (*P*) is shown in each panel. R^2_m represent the level of variance explained by the fixed factors on the figure.

Fig. 3 The effects of functional diversity (FD) of leaf thickness (a), functional diversity of leaf nitrogen (N) content (b), and community-weighted mean (CWM) of specific leaf area (SLA, c) on litterfall production. The figures were plotted based on the linear-mixed effect models reported in Table 1. Blue lines and grey shaded areas represent fitted regressions and their bootstrapped 95% confidence intervals. R^2_m represent the level of variance explained by the fixed factors on the figure. (d) The structural equation model depicting the direct and indirect effects of tree species richness, CWM of SLA, and leaf area index (LAI) on litterfall production. Fisher's C = 1.47, df = 2, P = 0.479. Blue and red arrows represent positive and negative relationships, respectively. The number beside the arrow is the corresponding standardized coefficient (*r*), and the significance level is set at α=0.05. $^*P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. R^2_m and R^2_c represent the level of variance of the variable explained by all paths from the fixed effects and both the fixed and random effects ("species"), respectively.

Fig. 4 Structural equation model depicting the direct and indirect influences of tree species richness, litterfall production, forest floor decay rate, litter species richness, soil temperature and soil moisture on forest floor mass. Fisher's C = 17.16, df = 18, P = 0.512. The single-headed arrow represents the directional influence of one variable on another. Blue and red arrows represent positive and negative relationships, respectively. The number beside the arrow is the corresponding standardized coefficient (r), and the significance level is set at α=0.05. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. R^2_m and R^2_c represent the level of deviance of the variable explained by all paths from the fixed effects and both the fixed and random effects ("species"), respectively.

Fig. 5 The effects of litterfall production (a) and litter species richness (b) on the forest floor decay rate. The results are based on the following linear mixed-effect model: $Y = \beta_0+\beta_1\times\text{Litterfall}+\beta_2\times\text{LiSR}+\beta_3\times\text{ST}+\beta_4\times\text{SM}+\pi_{species}+\varepsilon$. Litterfall is the annual litterfall production; LiSR, litter species richness; ST, soil temperature; SM, soil moisture. Blue lines and grey shaded areas represent fitted regressions and their bootstrapped 95% confidence intervals. The significance value (P) is shown in each panel. R^2_m represent the level of variance explained by the fixed factors on the figure.

Fig. 6 The effects of CWM of leaf nitrogen (N) content (a) and CWM of leaf dry matter content (LDMC, b) on the forest floor mass. The results are based on the linear-mixed effect models reported in Table 1. Blue lines and grey shaded areas represent fitted regressions and their bootstrapped 95% confidence intervals. The significance value (P) is shown in each panel. R^2_m represent the level of variance explained by the fixed factors on the figure.

Supporting information

Table S1 The 32 tree species name list, abbreviation, and leaf type category in this study.

Table S2 Tree growth in each tree species composition treatment.

Table S3 The Akaike information criterion (AIC) values for the full model (Eqn. 2 in Methods) and the most parsimonious model, and the effects (*P* value) of intercept, tree species richness (TSR), soil pH, soil carbon to nitrogen ratio (C/N), soil temperature (ST), and soil moisture (SM) of the most parsimonious models (n=283).

Table S4 The effects of functional diversity (FD) and community-weighted mean (CWM) of leaf litter traits on forest floor decay rate.

Fig. S1 Layout of the experimental plots (Left) and the pictures of the growth of trees taken in August 2022 (Right).

Fig. S2 The litter collector and the diagram of litter collectors placed in monocultures, 4-, 8-, 16-, and 32-species mixtures.

Fig. S3 Conceptual models showing the possibilities of direct and indirect effects of tree species richness on litterfall production and forest floor mass .

Fig. S4 Monthly litterfall production at the tree species richness levels of 1, 4, 8, 16, and 32 between April 2021 and August 2022.

Fig. S5 The interactive effects of tree species richness and functional diversity of leaf thickness (Lthick), functional diversity of leaf nitrogen (N), and community-weighted mean (CWM) of specific leaf area (SLA) on the litterfall production.

Fig. S6 The effects of tree species richness on the litterfall production, forest floor decay rate, and forest floor mass (n=279, excluding the four plots with 32 species).

Fig. S7 The effects of functional diversity (FD) of leaf thickness , functional diversity of leaf nitrogen (N) content , and community-weighted mean (CWM) of specific leaf area (SLA) on litterfall production.

Fig. S8 Structural equation model depicting the direct and indirect influences of tree species richness, litterfall production, forest floor decay rate, litter species richness, soil temperature and soil moisture on forest floor mass (n=279, excluding the four plots with 32 species).

Fig. S9 The effects of litterfall production and litter species richness on the forest floor decay rate (n=279, excluding the four plots with 32 species).

Fig. S10 The effects of CWM of leaf nitrogen content (N), CWM of leaf dry matter content (LDMC) on the forest floor mass (n=279, excluding the four plots with 32 species).

Fig. S11 The effects of tree species richness on the litterfall production, forest floor decay rate, and forest floor mass (n=258, excluding the 16 and 32 species mixtures).

Fig. S12 The effects of functional diversity (FD) of leaf thickness , functional diversity of leaf nitrogen (N) content , and community-weighted mean (CWM) of specific leaf area (SLA) on litterfall production.

Fig. S13 Structural equation model depicting the direct and indirect influences of tree species richness, litterfall production, forest floor decay rate, litter species richness, soil temperature and soil moisture on forest floor mass (n=258, excluding the 16 and 32 species mixtures).

Fig. S14 The effects of litterfall production and litter species richness on the forest floor decay rate (n=258, excluding the 16 and 32 species mixtures).

Fig. S15 The effects of CWM of leaf nitrogen content (N), CWM of leaf dry matter content (LDMC) on the forest floor mass (n=258, excluding the 16 and 32 species mixtures).

Fig. S16 The relationship between litter species richness and community-weighted mean (CWM) of litter tannins content.