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RESEARCH ARTICLE

Moderate size diversity of tree roots has largest effect on the carbon loss in tropical soils

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Abstract

1. Most studies focused on the above-ground diversity on leaf litter decomposition and soil respiration in tropical ecosystems, however, the mechanism understanding of root diversity on decomposition is still unclear.
2. We selected the fine-roots of 21 dominant tree species from a tropical rainforest in Xishuangbannan, China, then conducted a 360-day and well-replicated (21 replications) incubation decomposition experiment with a 0, 1, 3, 6, 9, 12, 15, 18 and 21 species gradient of root diversity. The fine-root mass loss, CO_2 release, and their potential drivers were analysed.
3. The results showed that as fine-root diversity increased, soil properties, microbial diversity and fungal biomass changed nonlinearly, leading to higher litter mass loss and soil CO_2 release in the moderate diversity with 9, 12 and 15 species mixtures. Non-additive effect analysis also indicated that synergistic effects were greater in these three mixtures than others on soil CO_2 release and litter mass loss. The indirect effects of soil properties and microbial communities were larger than the direct effect of fine-root diversity.
4. Our findings suggest that fine-root diversity has nonlinear relationships with litter decomposition and soil CO_2 release in tropical forests, thus highlighting the importance of plant diversity due to its role in the carbon cycle under global change scenario.

KEYWORDS
biodiversity, fine-root, litter mass loss, soil CO_2 release, tropical forest

1 | INTRODUCTION

Plant diversity is decreasing due to global climate change and human activity (Cao et al., 2022; Ohashi et al., 2019), with environmental pressures on plants expected to increase in future, biodiversity loss worsens (Lehnert et al., 2019; Yang et al., 2019). Due to their large carbon (C) pools, tropical forests are particularly important to the global C budget (Barbier et al., 2020). Despite their high biodiversity and large C storage capacity (Gibbs et al., 2010; Pan et al., 2011), tropical forests are one of the most threatened ecosystems in the world (Schulz et al., 2019). Litter decomposition in forests plays a critical role in the global C cycle, which transfers nutrients to the soil and represents an important source of CO_2 entering the atmosphere (Gessner et al., 2010). Root-derived C is sequestered in the soil more efficiently than leaf-derived C and is thus more consequential for the global C cycle (Craig et al., 2022).

Although many studies focused on the decomposition of above-ground litter, the effects of root diversity and root litter decomposition on soil C cycling have been frequently overlooked in forest ecosystems (Canessa et al., 2022). Therefore, a better understanding of root diversity and its effect on soil C cycling is imperative in tropical rainforests.

Because root-derived C forms a larger portion of the relatively stable soil C pool than C originating in above-ground litter, root decomposition is an important driver of terrestrial C flux (Kätterer et al., 2011). Root diameter influences the chemical and physical properties of root litter and, subsequently, litter quality (Silver & Miya, 2001). Root decomposition appears to be particularly sensitive to soil conditions such as moisture, oxygen concentration, pH and inorganic nutrient limitation (Garcia-Palacios et al., 2013; See et al., 2019). Mixed litter can alter decomposition by its effects on litter quality (i.e. mass ratio hypothesis) and litter trait dissimilarity (i.e. complementary use) (Canessa et al., 2022; Zhang et al., 2023), the fine-roots contain different elements and substances, and their chemical properties vary significantly (Gessner et al., 2010; Prieto et al., 2016). The effects of mixed root litter on decomposition rate and nutrient release have not been well-studied (Man et al., 2020; Yang et al., 2022). Fine-roots (diameter < 2 mm) have higher nutrient contents and are regarded as high turnover rate in terrestrial ecosystems (McCormack et al., 2015), in which the fine-roots of many plant species die within a year of their formation (Fogel, 1985). The turnover of fine-root biomass accounts for c. 14%–27% of net primary production globally (McCormack et al., 2015) and is estimated to contribute 33% of annual litter inputs in forests around the world (Freschet et al., 2010). Fine-root litter input can result in faster rates of decomposition, which in turn appear to activate microorganisms to stabilise soil organic matter (Cotrufo et al., 2013). Compared with fine-root growth and production, the fine-root decomposition processes and mechanisms need to be better understood due to their important role in C dynamics of the forest below-ground.

Microorganisms transfer nutrients between different litter types within mixtures, which can help optimise resource availability for decomposers, and these effects may be stronger for slowly decomposing litter types within a mixture (Liu et al., 2020; Man et al., 2020). For example, mixed litter has positive effects on decomposition, largely driven by increased microbial diversity (Liu et al., 2020). Microbial decomposition converts the C contained within the detritus into CO_2 and releases nutrients for plant growth (Heijboer et al., 2018). The mechanisms underlying the non-additive effects of diverse mixtures of leaf and root litter on decomposition are still disputed, because different diversity and experimental durations yielded inconsistent results, such as antagonism and synergism effects in forest ecosystems (Gripp et al., 2018; Lecerf et al., 2011; Leppert et al., 2017). For example, a previous study suggested that litter species composition drives this effect and that the chemistry of composite litter may be the predominant factor under specific environmental conditions (Handa et al., 2014). In addition, soil fungi play critical and unique roles in terrestrial ecosystem processes, and fungi are better equipped to decompose complex litter (Tedersoo et al., 2014). Therefore, fungal communities were considered as the primary agents of decomposition (Glassman et al., 2018). However, there are knowledge gaps on how the relationship between fungi and fine-root diversity influences decomposition in tropical rainforests.

To understand how fine-root diversity affects litter decomposition in tropical rainforests, we presented a year-long laboratory experiment to elucidate the effects of fine-root diversity on litter decomposition. Since litter decomposition has an important effect on soil C cycling (McGuire & Treseder, 2010), soil CO_2 release was measured to analyse how the soil C cycle responded to fine-root diversity. We hypothesised that: (1) The decomposition of mixed root litter has a non-additive effect such that moderate but not highest diversity has the strongest effect on fine-root litter mass loss during decomposition. Since the mixed litters with different physical and chemical properties lead to various nutrient inputs, which can affect soil microbial community structure and its function (Chen et al., 2017; Gessner et al., 2010); (2) The pattern of soil CO_2 release would be similar to the decomposition of fine-root litter, while soil microbial community regulates the processes. Because the substances that are difficult to decompose within mixed litters would inhibit microbial activities (Gessner et al., 2010; Prieto et al., 2016; Sun et al., 2018).

2 | MATERIALS AND METHODS

2.1 | Materials

Fine-root litters (FRL) were collected in October 2018 and surface soil (0–20 cm) was collected in October 2020 in a tropical rainforest in Xishuangbanna (101°34′26″–47″ E, 21°36′42″–58″ N), Yunnan Province, China. This study did not need permission for fieldwork. For fine-root collection, 21 dominant tree species belong to 12 families and 17 genera were selected (Table 1). Each species of fine-roots was obtained from 4 randomly selected trees to make them enough for experiment. FRL was air-dried and sterilised with humid heat (121°C, 30 min, two successive sterilization treatments). For soil collection, five sampling locations were randomly chosen. The humus was removed from the soil surface, sampled soil was mixed into a single composite sample, the residual roots and stones were manually removed, and the soil was passed through a 2 mm sieve.

2.2 | Experimental design

A well-replicate incubation experiment was established with 9 treatments with 21 replicates in each treatment. They were coded with root species 0 (CK), 1 (M1), 3 (M3), 6 (M6), 9 (M9), 12 (M12), 15 (M15), 18 (M18) and 21(M21), respectively. Before we set the experiment, 21 kinds of fine-roots were labelled from No. 1 to

TABLE 1 Classification of 21 tropical tree species and their fine-root nutrient contents.

No.	Order	Family	Genus	Species	TC ($g\,kg^{-1}$)	TN ($g\,kg^{-1}$)	TP ($g\,kg^{-1}$)
1	Ranales	Annonaceae	*Pseuduvaria*	*Pseuduvaria indochinensis*	335.12	14.10	0.25
2			*Alphonsea*	*Alphonsea monogyna*	434.71	7.55	0.14
3		Myristicaceae	*Knema*	*Knema furfuracea*	406.43	11.69	0.14
4			*Myristica*	*Myristica yunnanensis*	401.03	9.95	0.14
5		Lauraceae	*Litsea*	*Litsea dilleniifolia*	400.78	16.18	0.15
6				*Litsea verticillata*	358.83	5.76	0.05
7	Euphorbiales	Euphorbiaceae	*Cleidion*	*Cleidion brevipetiolatum*	353.44	14.68	0.19
8			*Trigonostemon*	*Trigonostemon thyrsoideus*	344.08	13.01	0.11
9			*Baccaurea*	*Baccaurea ramiflora*	373.64	7.19	0.14
10	Urticales	Moraceae	*Ficus*	*Ficus auriculata*	402.50	5.56	0.12
11				*Ficus langkokensis*	390.93	5.20	0.09
12				*Ficus oligodon*	352.11	5.95	0.13
13	Parietales	Guttiferae	*Garcinia*	*Garcinia cowa*	423.26	7.13	0.11
14				*Garcinia lancilimba*	440.04	5.54	0.08
15		Dipterocarpaceae	*Parashorea*	*Parashorea chinensis*	426.46	8.21	0.14
16	Rutales	Meliaceae	*Chisocheton*	*Chisocheton paniculatus*	417.91	8.99	0.04
17			*Dysoxylum*	*Dysoxylum binectariferum*	383.03	12.58	0.02
18	Ebenales	Ebenaceae	*Diospyros*	*Diospyros nigrocortex*	404.95	10.74	0.11
19	Fagales	Fagaceae	*Castanopsis*	*Castanopsis hystrix*	398.05	13.62	0.19
20	Sapindales	Icacinaceae	*Pittosporopsis*	*Pittosporopsis kerrii*	411.90	4.85	0.06
21	Malvales	Elaeocarpaceae	*Elaeocarpus*	*Elaeocarpus varunua*	368.62	7.25	0.18

Abbreviations: AM, arbuscular mycorrhiza; EM, ectotrophic mycorrhiza; TC, total carbon; TN, total nitrogen; TP, total phosphorus.

No. 21. To exclude the effects caused by the identity of species rather than diversity, we set 21 replicates and ensured that each species can be included in the incubation experiment in our study. With the treatment of 1 species fine-root (i.e. M1), we chose each species of fine-root for one repeat. With the treatments of 3, 6, 9, 12, 15 and 18 species fine-roots, we started with No. 1 to No. 3, No. 6, No. 9, No. 12, No. 15 and No. 18, respectively, then rotations started from No. 2. For example, the treatment of 3 species fine-roots (M3), for the first repeat we chose roots No. 1, No. 2 and No. 3; for the second repeat we use roots No. 2, No. 3 and No. 4; for the third repeat we use roots No. 3, No. 4 and No. 5, and so on. For each treatment with one or different species, the average weight of fine-roots was weighted for each repeat; that is, with the treatment 21 of species fine-roots, 0.1429 g for each species of fine-root was weighted and added 3 g to the bottle. Then, these 3 g of FRL were added to each 500 mL culture bottle with added 100 g of dry soil for each. The fine-roots were mixed with the soil. In total, there were 189 bottles in our study. Soil moisture in the bottle was held at 60% and sustained during experiment. In line with the dynamics of seasonal temperature in tropical Xishuangbanna, the bottles were incubated in the dark at 25°C, 21.5°C and 18°C for 120 days for each temperature. Soil CO_2 release was measured every month and residual FRL was recovered at the end of the experiment.

2.3 | Sample measurements

Nutrient elements were measured in FRL and soil of 21 plants prior to the beginning of the experiment. After 360 days of incubation, a total of 189 soil samples were measured. A portion of the soil was used for the nutrient analysis, and the remainder was frozen at −80°C for fungal community and soil microbial phospholipid fatty acid (PLFA) analysis. All soil samples of ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and dissolved organic carbon (DOC) were extracted with 0.5 $mol\,L^{-1}$ K_2SO_4. Soil available phosphorus (AP) was extracted with 0.5 $mol\,L^{-1}$ $NaHCO_3$ solution (pH = 8.5), total nitrogen (TN) and total phosphorus (TP) were determined with sodium salicylate and molybdenum antimony methods, respectively. SOC and DOC were determined using a merged Vario TOC analyser (Vario TOC, Langenselbold, Germany); SOC was determined by dry combustion at 980°C in solid mode, and DOC was determined in liquid mode. The concentrations of TN, NH_4^+-N, NO_3^--N, TP and AP were determined through auto discrete analyser (De Chem-Tech. GmbH, CleverChem380, Hamburg Germany). Soil C to N ratio (C:N) was calculated as the ratio of SOC to TN. Soil pH in water was measured using pH meter, and the soil-to-solution ratio (v/v) was 1:2.5.

Fresh soil was used to determine soil microbial PLFA and fungal community composition. Soil bacterial and fungal biomass was characterised by PLFA analysis (Chen et al., 2022). The PLFAs were considered as bacterial indicators (i14:0, a15:0, i15:0, i16:0, a17:0, i17:0, 16:1ω7c, 16:1ω9c, cy17:0, 17:1ω8c, 18:1ω7c, 18:1ω9c and cy19:0) and fungal indicator (18:2ω6c). Fungal communities were profiled by sequencing amplicons targeting the fungal ITS sequence of the 18S rRNA gene using an Illumina MiSeq platform (San Diego, CA, USA). Fungal diversity was assessed using the Shannon, Simpson, Chao1 and Pielou-e indices. Pairwise mean Bray-Curtis dissimilarities were calculated using the 'vegan' package in R statistical software. We also defined and compared fungal functional guilds using the FUNGuild database. To collect gas samples, culture bottles were sealed and placed back in the incubator for 2h. A syringe was used to collect and transfer gas to 12mL exetainers and CO_2 concentration was measured using a gas chromatograph (GC-2014, Shimadzu, Japan).

2.4 | Calculations and statistical analysis

We used linear regression to examine generalised patterns of microbial variables (microbial biomass and fungal diversity) and soil properties along the root diversity for different FRL diversity and litter mass loss values. Based on the values obtained from single species litter, we calculated the expected values (E) of each soil CO_2 release rate and litter mass loss (Wardle et al., 1997):

$$E = \sum_{i=1}^{S} R_i / S, \tag{1}$$

where R_i is the soil response variable when only species i is included and S denotes the number of species in each litter mixture. For each FRL mixture, we determined the difference between observed values (O) and E via paired t-tests for non-additive effects in each response variable. For each response variable, a significant difference between O and E ($p<0.05$) indicated a non-additive effect; otherwise, an additive effect was inferred. The direction and magnitude of non-additive effects (or litter mixture effects, LME) were calculated as the following equation:

$$\mathrm{LME} = \left[(O - E)/E\right] \times 100\%, \tag{2}$$

wherein, positive values ($O-E$) indicate synergistic effects, and negative values ($O-E$) indicate antagonistic effects.

Soil CO_2 release rate was calculated as the following equation:

$$RR = M/V_m \times 460\left(C_2 - C_1\right) \times 1/1000\,\mathrm{m} \times P/P_0 \times T_0/T, \tag{3}$$

where RR is CO_2 emission (mg (CO_2) g^{-1} soil h^{-1}), M is the molar mass of CO_2 (44 g mol^{-1}), V_m is the molar volume of 22.4 (L mol^{-1}), 460 is the gas volume to be measured in the culture bottle (mL), C_1 is the concentration of the gas to be measured (ppm) in the container, C_2 is the concentration of the gas to be measured (ppm) in the container for every hour; m is the mass of dry soil used in the experiment (g), P is atmospheric pressure in Kunming, China, 80.735 kpa, P_0 is standard atmospheric pressure of 101.325 kpa, T_0 is absolute temperature under standard conditions of 273.15K and T is the absolute experimental temperature (273.15 + T (°C)) K. Litter mass loss was calculated as the following equation:

$$ML = \left(m_0 - m_1\right)/m_0 \times 100\%, \tag{4}$$

where m_0 is the initial FRL dry weight and m_1 is the dry weight of FRL at the end of the experimental period. Since addition of different litter species would change soil physico-chemical properties and microbial community structure, which would affect the rate of microbial respiration and litter decomposition (Hu et al., 2016). We used a piecewise structural equation model (SEM) to assess the effects of FRL diversity on the biomass, diversity, and composition of fungal communities via changes in soil abiotic variables (Lefcheck, 2016). The priori SEM was designed based on the fact that different litter addition to the soil would change the physical and chemical properties and microbial community structure of soil, which would change soil CO_2 release and litter decomposition (Hu et al., 2016; Man et al., 2020). Differences in soil properties, CO_2 release, microbial PLFAs, fungal alpha diversity and fungal trophic mode relative abundance were compared using ANOVA and least significant difference methods. All statistical analyses were performed with R version 3.6.3 (R Core Team, 2017).

3 | RESULTS

3.1 | Effects of FRL diversity on the decomposition and soil CO_2 release

The mass loss of FRL was nonlinearly correlated with FRL diversity (Figure 1a). Treatment M9 had the maximum and treatment M3 had the minimum of FRL mass losses (Table S1). There was a nonlinear correlation between soil CO_2 release and FRL diversity (Figure 1; Figure S1). With increasing FRL diversity, CO_2 release first increased and then decreased, and M9, M12 and M15 had the highest rates of CO_2 release (Figure 1b). There was a positive relationship between soil CO_2 release rate and FRL mass loss ($p=0.012$) (Figure 1c). In addition, soil CO_2 release rate was positively correlated with soil C:N ratio ($p=0.035$, Figure 1d), negatively correlated with soil N:P ratio ($p=0.016$, Figure 1e), but uncorrelated with soil C:P ratio (Figure 1f). Three-way ANOVAs showed that FRL diversity, incubation time, incubation temperature and their interactions had significant effects on soil CO_2 release rate (Table S2).

3.2 | Effects of FRL diversity on soil properties

The FRL diversity had different effects on soil properties (Figure 2; Figure S2; Table S3). With increasing FRL diversity, SOC and NH_4^+-N concentrations increased (Figure 2a,d), DOC and NO_3^--N concentrations and soil C:N ratio first decreased and then increased (Figure 2b,e,i), but TN, AP and pH responded oppositely (Figure 2c,g,h). The mass loss of FRL was positively correlated with SOC, TN, DOC, NH_4^+-N and NO_3^--N and negatively correlated with AP, pH and C:N, but not significantly correlated with TP (Figure S3). Three-way ANOVAs showed

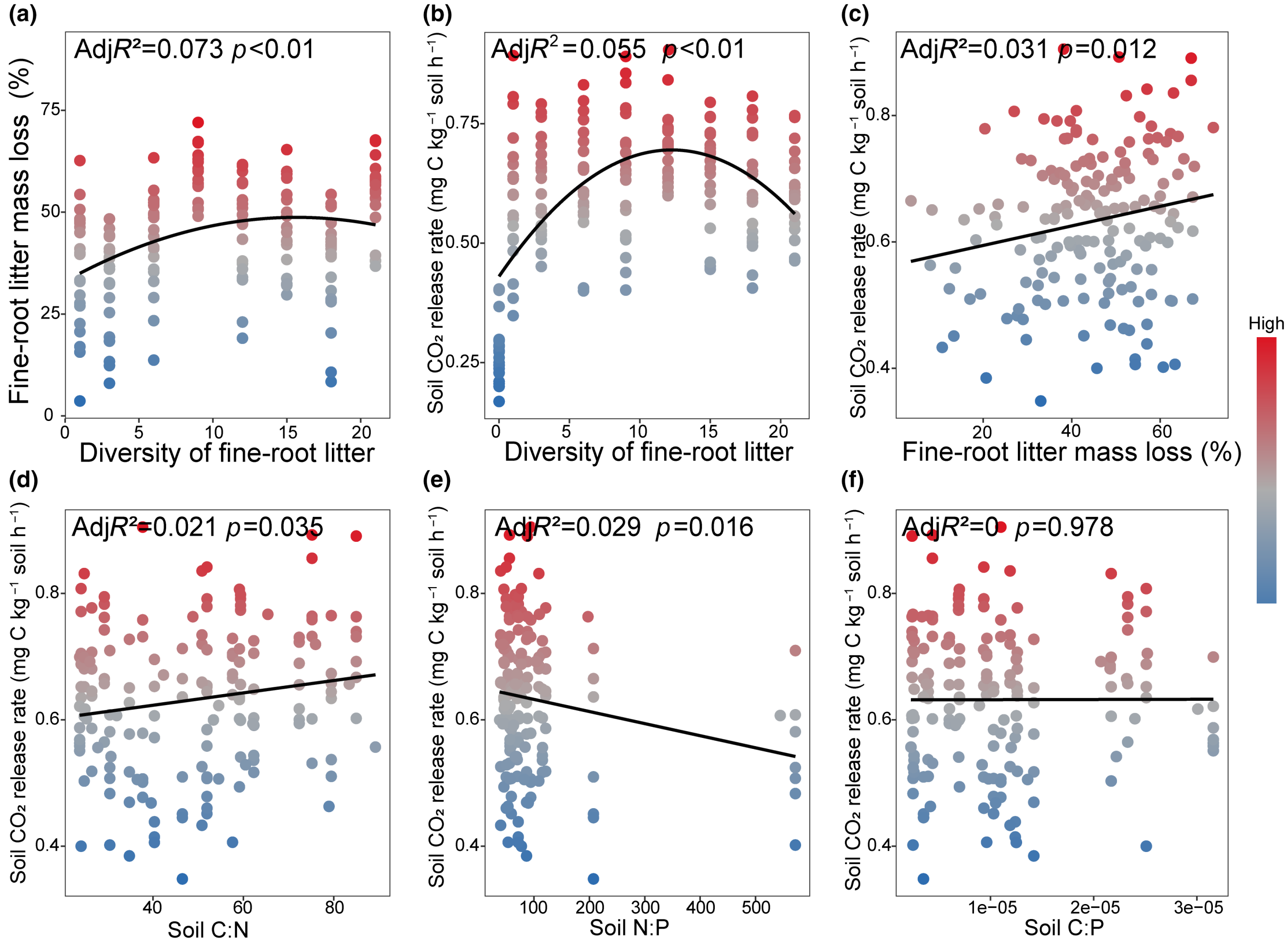


FIGURE 1 The relationships between fine-root litter diversity, mass loss and soil CO_2 release (a–c) and the effect of soil stoichiometry on soil CO_2 release rate (d–f).

that the added C, N and P content of FRL affected soil properties and microorganisms, and soil CO_2 release and FRL mass loss are mainly affected by the added C content of FRL (Table S3).

With increasing FRL diversity, soil total PLFAs first decreased and then increased, while fungal PLFAs and beta diversity exhibited the opposite trend (Figure 3a,b,f). FRL diversity had no significant effect on bacterial PLFAs or on the ratio of F:B (Figure 3c,d), but it was negatively correlated with fungal alpha diversity (Figure 3e). In M21, bacterial and fungal PLFAS increased significantly, as did PLFAs for Gram (−) and Gram (+) bacteria (Figure S4B–E). FRL mass loss was not correlated with fungal or bacterial PLFAs, the ratio of F:B, or fungal alpha diversity, but it was negatively correlated with fungal beta diversity (Figures S5 and S6). Soil fungal community composition was mainly affected by TN and pH, followed by AP, SOC, DOC, NO_3^--N and NH_4^+-N. TP was the only variable that did not have a significant effect on fungal community composition (Figure S3A). Moderate size diversity resulted in higher Chao1, Simpson, Shannon and Pielou-e indices of soil fungi (Figure S7). Bray-Curtis dissimilarities of soil fungi were significantly correlated with TN and pH (Figure S3B). The increased FRL diversity enhanced the relative abundance of Acomycota and decreased the relative abundance of Basidiomycota at the phylum level (Figures S8 and S9). Increased FRL diversity had no significant effect on saprotrophic fungi, but reduced Pathotroph-Saprotrph-Symbiotroph and Pathotroph-Symbiotroph fungi (Table S4; Figures S8–S10).

3.3 | Linking FRL mixed decomposition with soil properties

The FRL diversity had a synergistic effect on soil CO_2 release and FRL mass loss. M12 had the largest mixture effect on CO_2 release, while M21 had the smallest effect. The mixture effect values of M12 and M15 were significantly higher than M3 (Figure 4a). The mixture effect value of M9 was the largest, while M3 and M18 had the smallest FRL mass loss. The mixture effect values of M21 and M12 were significantly different from other treatments (Figure 4b).

Piecewise SEM shows that the change in soil properties significantly affected soil CO_2 release and soil microorganisms, and soil microorganism PLFAs significantly affected soil CO_2 release (Figure 5a). The direct effect of FRL diversity on soil CO_2 release was lower than the indirect effects of soil properties, soil microbial PLFAs and soil

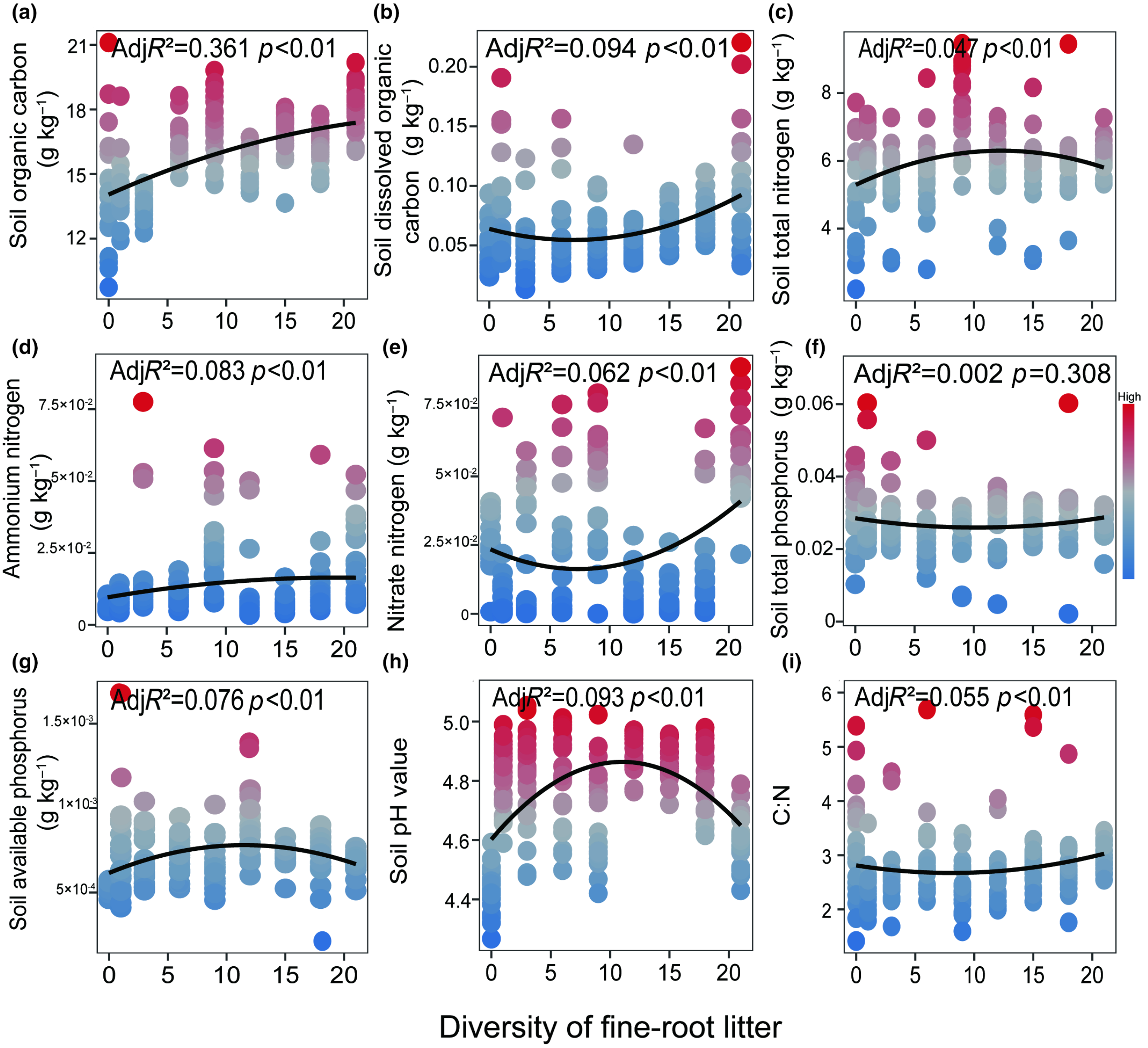


FIGURE 2 Effects of fine root litter diversity on soil properties, i.e. soil organic carbon (a), dissolved orgnic carbon (b), total nrogen (c), ammonium nitrogen (d), nitrate nitrogen (e), soil total phosphorus (f), soil available phosphorus (g), soil pH (h) and carbon to nitrogen ratio (i).

fungi (Figure 5b). Both soil properties and soil microbial PLFAs significantly affected FRL mass loss (Figure 5c). The relationship between FRL mass loss and FRL diversity was determined by the indirect effects of soil properties, soil microbial PLFAs and soil fungi (Figure 5d).

4 | DISCUSSION

4.1 | Effects of fine-root diversity on litter mass loss

We investigated decomposition along a fine-root diversity gradient and found that litter mass loss showed a non-additive effect and moderate fine-root diversity had a greater positive effect on litter mass loss (Figures 1 and 5). The mixture effects of decomposition with M9 and M12 were larger than other treatments, which was consistent with our first hypothesis. The first explanation would be that the different litter inputs changed soil nutrients (Chen et al., 2017; Gessner et al., 2010), the trade-off effect on soil nutrients would regulate decomposition. Since soil nutrients can strongly affect litter decomposition through various pathways (Wan et al., 2022; Wu et al., 2019). The evidence was that SOC, TN, NH_4^+-N and NO_3^--N were positively correlated with litter mass loss, while soil TP, AP and pH negatively correlated with litter mass loss in our study. The greatest loss of litter mass with moderate fine-root diversity implied the largest synergistic effect. The combination of different litter species can alter the process of decomposition via multiple non-exclusive mechanisms, leading to either an acceleration or deceleration in

FIGURE 3 Effects of fine root litter diversity on soil microorganisms, i.e. total PLFAs (a), fungal PLFAs (b), bacterial PLFAs (c), fungi to bacteria ratio (d), fungal alpha diveristy (e) and fungal beta diversity (f).

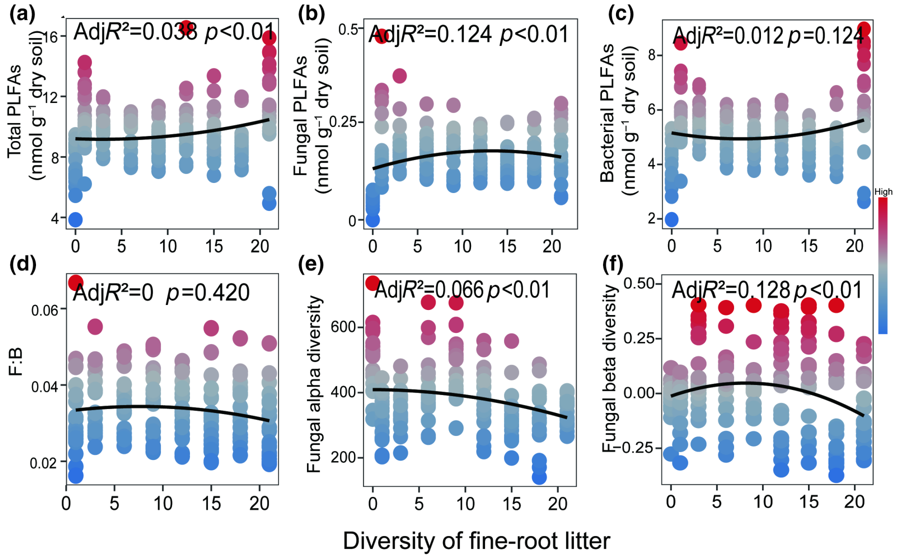
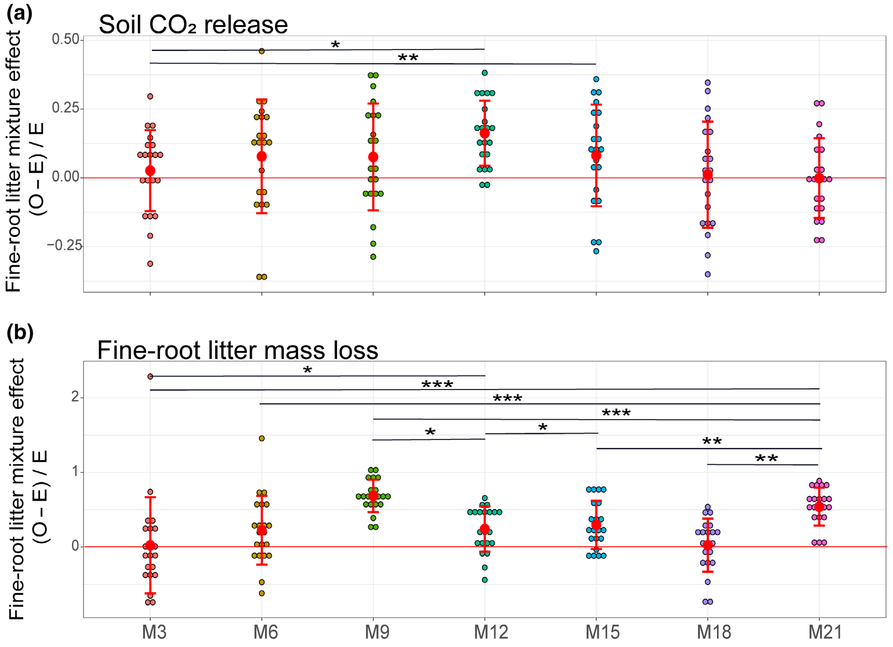


FIGURE 4 Mixture effects on the soil CO_2 release (a) and decomposition of fine root litter (b). *O*: observed values, *E*: expected values. M3: 3 kinds of fine-root litter, M6: 6 kinds of fine-root litter, M9: 9 kinds of fine-root litter, M12: 12 kinds of fine-root litter, M15: 15 kinds of fine-root litter, M18: 18 kinds of fine-root litter, M21: 21 kinds of fine-root litter. Values are means ± *SE*. Asterisks indicate significant deviations from zero (Student's t tests; * <0.05, ** <0.01, *** <0.001).



the mass loss of litter mixtures or individual components (Gessner et al., 2010).

Second, soil microbial community would be another important driver affecting decomposition (Gessner et al., 2010; Lecerf et al., 2011; Sheng et al., 2019). We found that fine-root diversity was significantly correlated with both microbial biomass and fungal diversity and moderate fine-root litter diversity led to the maximum fungal diversity (Figure 3; Figure S4). Recent work found that the transition from mono- to mixed-species plant litter could increase decomposition by 34.7% in forest ecosystems (Mori et al., 2020), which supported our findings. Our study indicated that moderate fine-root diversity can create positive feedback between mass loss and fungal activity, and may explain why the highest diversity was not associated with the highest litter mass loss. Since high diversity with various identities can also increase substances that are difficult to decompose and inhibit microbial activities (Man et al., 2020; Prieto et al., 2016, 2017). Previous work has shown that higher diversity in forested systems will lead to antagonistic effects and decrease litter decomposition (Silver & Miya, 2001), supporting our findings. Furthermore, we found that fine-root diversity had no significant effect on the trophic mode of saprotrophic fungi, but increased their relative abundance. For example, M9 treatment increased the abundance of fungi belonging to the Sordariales order, which can stimulate the decomposition of organic matter (Zhou et al., 2021).

4.2 | Effects of fine-root litter diversity on soil CO_2 release

Our finding indicated that fine-root addition promoted soil CO_2 release, and moderate fine-root diversity was associated with higher

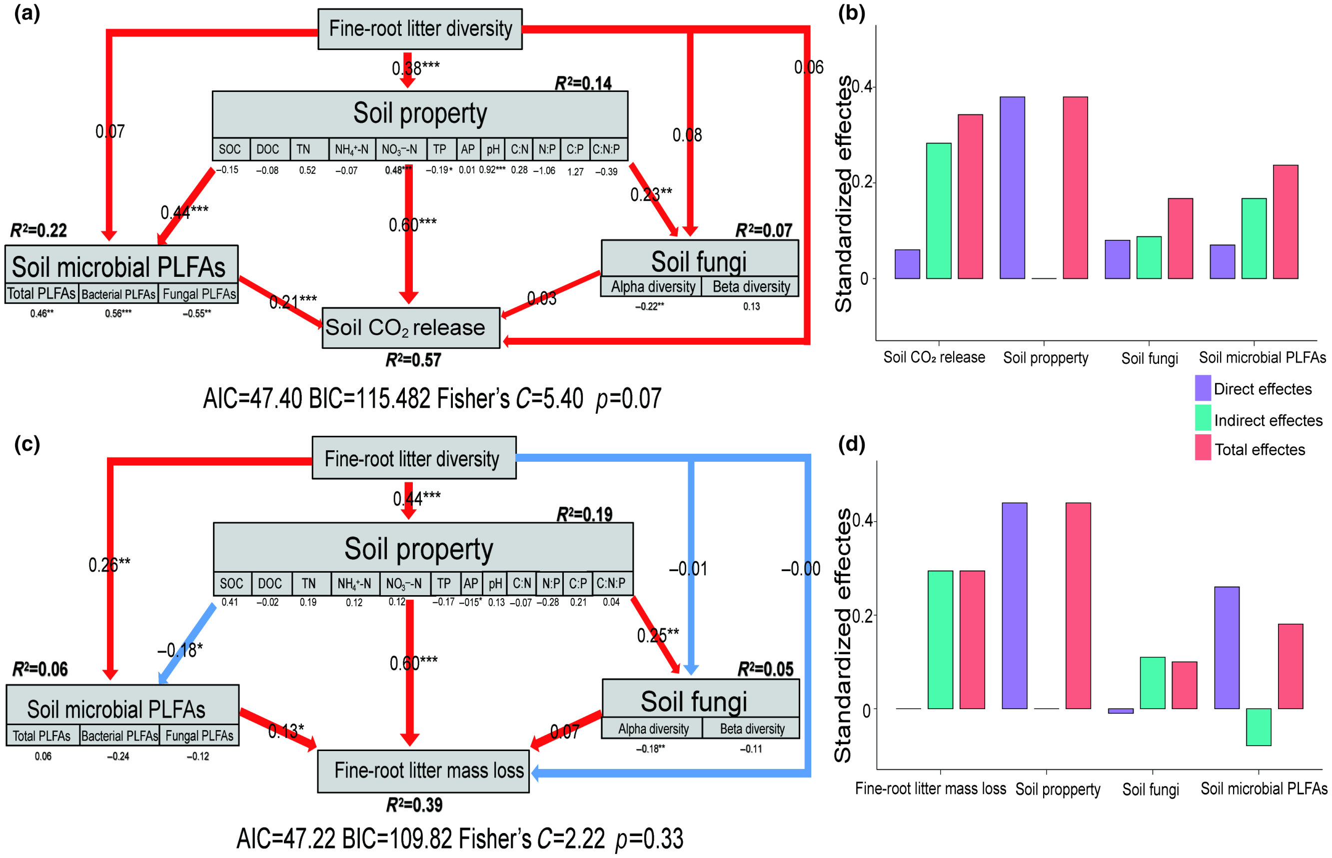


FIGURE 5 Structural equation model (SEM) of the effects of fine root litter diversity on soil CO_2 release (a, b) and fine root litter mass loss (c, d). AP, available phosphorus; C:N, ratio of SOC to TN; C:N:P, ratio of SOC, TN and TP; C:P, ratio of SOC to TP; DOC, soil dissolved organic carbon; N:P, ratio of TN to TP; NH_4^+-N, ammonia nitrogen; NO_3^--N, nitrate nitrogen; SOC, soil organic carbon; TN, soil total nitrogen; TP, total phosphorus.

soil CO_2 release (Figures 1 and 5). Consistent with the second hypothesis, the release of soil CO_2 mirrored the decomposition process, the highest decomposition at moderate diversity of can promote the release of soil CO_2 in this study. Furthermore, the whole effect of mixed fine-root litter on CO_2 release was a synergistic effect, probably because the leaching and transfer of nutrients and inhibitory compounds between litter species can result in synergistic effect with litter mixtures (Handa et al., 2014). A recent study suggested that high species diversity of root litter reduces the cumulative release of soil CO_2 (Man et al., 2020), which partially supported our findings that highest diversity had not highest CO_2 release. The marginal antagonistic effect on cumulative soil CO_2 release in mixtures was likely the result of differences in the species composition of root litter. For example, litter with high C:N ratio or high lignin, tannin or polyphenol contents increased antagonism, influencing cumulative soil CO_2 (Gessner et al., 2010).

Moderate fine-root diversity had the highest soil CO_2 release, implying that litter decomposition and associated nutrient release can provide substrates for microbes (Liu et al., 2019; Tan et al., 2021). Because moderate fine-root diversity increased microbial activities and leads to accelerated organic C mineralization through the priming effect (Heijboer et al., 2018), thus enhanced soil CO_2 release in the tropical rainforest. As mentioned above, M9 treatment was associated with a relatively high abundance of saprophytic fungi. Higher microbial biomass and activities have an obvious relationship with soil respiration (Wu et al., 2017). A previous experiment also found that litter removal reduced the diversity of fungi, especially saprotrophic fungi, thus reduced soil respiration (Zhou et al., 2021). Taking into account the nonlinear dynamics in our incubation experiment, the effect of fine-root diversity on soil C cycling should be given adequate attention in those forests with high plant diversity in future.

5 | CONCLUSIONS

Our one-year-long incubation experiment showed that there was a strong correlation between fine-root diversity and litter mass loss and soil CO_2 release, with moderate diversity of fine-root (i.e. 9, 12 and 15 species mixtures) having the greater effects. The potential mechanisms would be that different root diversity resulted in different releases of nutrients and changed soil microbial biomass and fungal community composition such as higher relative abundance of saprophytic fungi in moderate fine-root diversity. Changes in soil physico-chemical properties and microbial community composition directly and indirectly affected litter mass loss and soil CO_2 release, and the effects of soil properties on both litter mass loss and soil CO_2 release were higher than those of microorganisms. In summary, our findings suggest that fine-root diversity has nonlinear relationships with litter decomposition and soil CO_2 release in tropical forests, thus, the appropriate plant diversity must be sustained under global change scenario.

AUTHOR CONTRIBUTIONS

Jianping Wu and Deyun Chen designed the experiment and wrote the manuscript. Deyun Chen, Shangwen Xia, Sicheng Li, Shuting Zhang, Hong Chen and Xiaoyue Ding conducted the fieldwork and laboratory work. Jianping Wu and Deyun Chen took part in statistical analysis. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

All authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Zenodo (https://doi.org/10.5281/zenodo.7825769).

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REFERENCES

Barbier, E. B., Lozano, R., Rodriguez, C. M., & Troeng, S. (2020). Adopt a carbon tax to protect tropical forests. *Nature*, *578*(7794), 213–216. https://doi.org/10.1038/d41586-020-00324-w

Canessa, R., van den Brink, L., Berdugo, M. B., Hättenschwiler, S., Rios, R. S., Saldana, A., Tielborger, K., & Bader, M. Y. (2022). Trait functional diversity explains mixture effects on litter decomposition at the arid end of a climate gradient. *Journal of Ecology*, *110*(9), 2219–2231. https://doi.org/10.1111/1365-2745.13946

Cao, X., Tian, F., Herzschuh, U., Ni, J., Xu, Q., Li, W., Zhang, Y., Luo, M., & Chen, F. (2022). Human activities have reduced plant diversity in eastern China over the last two millennia. *Global Change Biology*, *28*(16), 4962–4976. https://doi.org/10.1111/gcb.16274

Chen, D., Cai, M., Li, D., Yang, S., & Wu, J. (2022). Response of soil organic carbon stock to bryophyte removal is regulated by forest types in southwest China. *Forests*, *13*(12), e2125. https://doi.org/10.3390/f13122125

Chen, H., Mommer, L., van Ruijven, J., de Kroon, H., Fischer, C., Gessler, A., Hildebrandt, A., Scherer-Lorenzen, M., Wirth, C., Weigelt, A., & Wurzburger, N. (2017). Plant species richness negatively affects root decomposition in grasslands. *Journal of Ecology*, *105*, 209–218. https://doi.org/10.1111/1365-2745.12650

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/gcb.12113

Craig, M. E., Geyer, K. M., Beidler, K. V., Brzostek, E. R., Frey, S. D., Grandy, A. S., & Richard, P. P. (2022). Fast-decaying plant litter enhances soil carbon in temperate forests but not through microbial physiological traits. *Nature Communications*, *13*(1), 1229. https://doi.org/10.1038/s41467-022-28715-9

Fogel, R. (1985). Root as primary produces in belowground ecosystems. In A. H. Fitter (Ed.), *Biological interactions in soil* (pp. 23–36). Blackwell Scientific Publications.

Freschet, G. T., Cornelissen, J. H. C., van Logtestijn, R. S. P., & Aerts, R. (2010). Evidence of the 'plant economics spectrum' in a subarctic flora. *Journal of Ecology*, *98*(2), 362–373. https://doi.org/10.1111/j.1365-2745.2009.01615.x

Garcia-Palacios, P., Maestre, F. T., Kattge, J., & Wall, D. H. (2013). Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecology Letters*, *16*(11), 1045–1053. https://doi.org/10.1111/ele.12179

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H., & Stephan, H. (2010). Diversity meets decomposition. *Trends in Ecology & Evolution*, *25*(6), 372–380. https://doi.org/10.1016/j.tree.2010.01.010

Gibbs, H. K., Ruesch, A. S., Achard, F., Clayton, M. K., Holmgren, P., Ramankutty, N., & Foley, J. A. (2010). Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(38), 16732–16737. https://doi.org/10.1073/pnas.0910275107

Glassman, S. I., Weihe, C., Li, J., Albright, M. B. N., Looby, C. I., Martiny, A. C., Treseder, K. K., Allison, S. D., & Martiny, J. B. H. (2018). Decomposition responses to climate depend on microbial community composition. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(47), 11994–11999. https://doi.org/10.1073/pnas.1811269115

Gripp, A. R., Esteves, F. A., Carneiro, L. S., Guariento, R. D., Figueiredo-Barros, M. P., Coq, S., Milcu, A., & Caliman, A. (2018). Weak to no effects of litter biomass and mixing on litter decomposition in a seasonally dry tropical forest. *Pedobiologia*, *68*, 20–23. https://doi.org/10.1016/j.pedobi.2018.02.003

Handa, I. T., Aerts, R., Berendse, F., Berg, M. P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M. O., Jabiol, J., Makkonen, M., McKie, B. G., Malmqvist, B., Peeters, E. T., Scheu, S., Schmid, B., van Ruijven, J., Vos, V. C., & Hattenschwiler, S. (2014). Consequences of biodiversity loss for litter decomposition across biomes. *Nature*, *509*(7499), 218–221. https://doi.org/10.1038/nature13247

Heijboer, A., de Ruiter, P. C., Bodelier, P. L. E., & Kowalchuk, G. A. (2018). Modulation of litter decomposition by the soil microbial food web under influence of land use change. *Frontiers in Microbiology*, *9*, 2860. https://doi.org/10.3389/fmicb.2018.02860

Hu, X., Liu, L., Zhu, B., Du, E., Hu, X., Li, P., Zhou, Z., Ji, C., Zhu, J., Shen, H., & Fang, J. (2016). Asynchronous responses of soil carbon dioxide, nitrous oxide emissions and net nitrogen mineralization to enhanced fine root input. *Soil Biology & Biochemistry*, *92*, 67–78. https://doi.org/10.1016/j.soilbio.2015.09.019

Kätterer, T., Bolinder, M. A., Andrén, O., Kirchmann, H., & Menichetti, L. (2011). Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agriculture, Ecosystems and Environment*, *141*(2), 184–192. https://doi.org/10.1016/j.agee.2011.02.029

Lecerf, A., Marie, G., Kominoski, J. S., LeRoy, C. J., Bernadet, C., & Swan, C. M. (2011). Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition. *Ecology*, *92*(1), 160–169. https://doi.org/10.1890/10-0315.1

Lefcheck, J. S. (2016). PIECEWISESEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579. https://doi.org/10.1111/2041-210X.12512

Lehnert, S. J., Kess, T., Bentzen, P., Kent, M. P., Lien, S., Gilbey, J., Clément, M., Jeffery, N. W., Waples, R. S., & Bradbury, I. R. (2019). Genomic signatures and correlates of widespread population declines in salmon. *Nature Communications*, *10*, 2996. https://doi.org/10.1038/s41467-019-10972-w

Leppert, K. N., Niklaus, P. A., & Scherer-Lorenzen, M. (2017). Does species richness of subtropical tree leaf litter affect decomposition, nutrient release, transfer and subsequent uptake by plants? *Soil Biology and Biochemistry*, *115*, 44–53. https://doi.org/10.1016/j.soilbio.2017.08.007

Liu, J., Liu, X., Song, Q., Compson, Z. G., LeRoy, C. J., Luan, F., Wang, H., Hu, Y., & Yang, Q. (2020). Synergistic effects: A common theme in mixed-species litter decomposition. *New Phytologist*, *227*(3), 757–765. https://doi.org/10.1111/nph.16556

Liu, X., Chen, S., Yang, Z., Lin, C., Xiong, D., Lin, W., Xu, C., Chen, G., Xie, J., Li, Y., & Yang, Y. (2019). Will heterotrophic soil respiration be more sensitive to warming than autotrophic respiration in subtropical forests? *European Journal of Soil Science*, *70*(3), 655–663. https://doi.org/10.1111/ejss.12758

Man, J., Tang, B., Xing, W., Wang, Y., Zhao, X., & Bai, Y. (2020). Root litter diversity and functional identity regulate soil carbon and nitrogen cycling in a typical steppe. *Soil Biology and Biochemistry*, *141*, 107688. https://doi.org/10.1016/j.soilbio.2019.107688

McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., Helmisaari, H. S., Hobbie, E. A., Iversen, C. M., Jackson, R. B., Leppalammi-Kujansuu, J., Norby, R. J., Phillips, R. P., Pregitzer, K. S., Pritchard, S. G., Rewald, B., & Zadworny, M. (2015). Redefining fine-roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist*, *207*(3), 505–518. https://doi.org/10.1111/nph.13363

McGuire, K. L., & Treseder, K. K. (2010). Microbial communities and their relevance for ecosystem models: Decomposition as a case study. *Soil Biology and Biochemistry*, *42*, 529–535. https://doi.org/10.1016/j.soilbio.2009.11.016

Mori, A. S., Cornelissen, J. H. C., Fujii, S., Okada, K., & Isbell, F. (2020). A meta-analysis on decomposition quantififies afterlife effects of plant diversity as a global change driver. *Nature Communications*, *11*(1), 4547. https://doi.org/10.1038/s41467-020-18296-w

Ohashi, H., Hasegawa, T., Hirata, A., Fujimori, S., Takahashi, K., Tsuyama, I., Nakao, K., Kominami, Y., Tanaka, N., Hijioka, Y., & Matsui, T. (2019). Biodiversity can benefit from climate stabilization despite adverse side effects of land-based mitigation. *Nature Communications*, *10*, 5240. https://doi.org/10.1038/s41467-019-13241-y

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., Phillips, O. L., Shvidenko, A., Lewis, S. L., Canadell, J. G., Ciais, P., Jackson, R. B., Pacala, S. W., McGuire, A. D., Piao, S., Rautiainen, A., Sitch, S., & Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, *333*(6045), 988–993. https://doi.org/10.1126/science.1201609

Prieto, I., Birouste, M., Zamora-Ledezma, E., Gentit, A., Goldin, J., Volaire, F., & Roumet, C. (2017). Decomposition rates of fine roots from three herbaceous perennial species: Combined effect of root mixture composition and living plant community. *Plant and Soil*, *415*(1), 359–372. https://doi.org/10.1007/s11104-016-3163-z

Prieto, I., Stokes, A., & Roumet, M. (2016). Root functional parameters predict fine root decomposability at the community level. *Journal of Ecology*, *104*(3), 725–733. https://doi.org/10.1111/1365-2745.12537

R Core Team. (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Schulz, G., Schneider, D., Brinkmann, N., Edy, N., Daniel, R., Polle, A., Scheu, S., & Krashevska, V. (2019). Changes in trophic groups of protists with conversion of rainforest into rubber and oil palm plantations. *Frontiers in Microbiology*, *10*, e240. https://doi.org/10.3389/fmicb.2019.00240

See, C. R., Luke, M. M., Hobbie, S. E., Flores-Moreno, H., Silver, W. L., & Kennedy, P. G. (2019). Global patterns in fine root decomposition: Climate, chemistry, mycorrhizal association and woodiness. *Ecology Letters*, *22*(6), 946–953. https://doi.org/10.1111/ele.13248

Sheng, Y., Cong, J., Lu, H., Yang, L., Liu, Q., Li, D., & Zhang, Y. (2019). Broad-leaved forest types affect soil fungal community structure and soil organic carbon contents. *Microbiology*, *8*(10), e874. https://doi.org/10.1002/mbo3.874

Silver, W. L., & Miya, R. K. (2001). Global patterns in root decomposition: Comparisons of climate and litter quality effects. *Oecologia*, *129*(3), 407–419. https://doi.org/10.1007/s004420100740

Sun, T., Hobbie, S. E., Berg, B., Zhang, H., Wang, Q., Wang, Z., & Hattenschwiler, S. (2018). Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(41), 10392–10397. https://doi.org/10.1073/pnas.1716595115

Tan, Q., Si, J., He, Y., Yang, Y., Shen, K., Xia, T., Kang, L., Fang, Z., Wu, B., Guo, Y., & Han, X. (2021). Improvement of karst soil nutrients by arbuscular mycorrhizal fungi through promoting nutrient release from the litter. *International Journal of Phytoremediation*, *23*, 1244–1254. https://doi.org/10.1080/15226514.2021.1889966

Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N. S., Wijesundera, R., Villarreal-Ruiz, L., Vasco-Palacios, A. M., Thu, P. Q., Suija, A., Smith, M. E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Poldmaa, K., … Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213), 1256688. https://doi.org/10.1126/science.1256688

Wan, X., Yu, Z., Wang, M., Zhang, Y., & Huang, Z. (2022). Litter and root traits control soil microbial composition and enzyme activities in 28 common subtropical tree species. *Journal of Ecology*, *110*(12), 3012–3022. https://doi.org/10.1111/1365-2745.14009

Wardle, D. A., Bonner, K. I., & Nicholson, K. S. (1997). Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos*, *79*(2), 247–258. https://doi.org/10.2307/3546010

Wu, H., Xiang, W., Chen, L., Ouyang, S., Xiao, W., Li, S., Forrester, D. I., Lei, P., Zeng, Y., Deng, X., Zeng, L., & Kuzyakov, Y. (2019). Soil phosphorus bioavailability and recycling increased with stand age in Chinese fir plantations. *Ecosystems*, *23*(5), 973–988. https://doi.org/10.1007/s10021-019-00450-1

Wu, J., Qian, Z., Fan, Y., Yao, L., Zhang, Q., & Cheng, X. (2017). Does short-term litter input manipulation affect soil respiration and its carbon-isotopic signature in a coniferous forest ecosystem of central China? *Applied Soil Ecology*, *113*, 45–53. https://doi.org/10.1016/j.apsoil.2017.01.013

Yang, K., Zhu, J., Zhang, W., Zhang, Q., Lu, D., Zhang, Y., Zheng, X., Xu, S., & Wang, G. (2022). Litter decomposition and nutrient release from monospecific and mixed litters: Comparisons of litter quality, fauna and decomposition site effects. *Journal of Ecology*, *110*(7), 1673–1686. https://doi.org/10.1111/1365-2745.13902

Yang, X., Huang, Z., Dong, M., Ye, X., Liu, G., Hu, D., Tuvshintogtokh, I., Tumenjargal, T., & Cornelissen, J. H. C. (2019). Responses of community structure and diversity to nitrogen deposition and rainfall addition in contrasting steppes are ecosystem-dependent and dwarfed by year-to-year community dynamics. *Annals of Botany*, *124*, 461–469. https://doi.org/10.1093/aob/mcz098

Zhang, W., Fornara, D., Yang, H., Yu, R., Callaway, R. M., & Li, L. (2023). Plant litter strengthens positive biodiversity-ecosystem functioning relationships over time. *Trends in Ecology & Evolution*, *38*, 473–484.

Zhou, Y., Sun, B., Xie, B., Feng, K., Zhang, Z., Zhang, Z., Li, S., Du, X., Zhang, Q., Gu, S., Song, W., Wang, L., Xia, J., Han, G., & Deng, Y. (2021). Warming reshaped the microbial hierarchical interactions. *Global Change Biology*, *27*(24), 6331–6347. https://doi.org/10.1111/gcb.15891

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Experimental design and fine-root litter mass loss (±SE).

Table S2: Fine-root litter diversity (Diversity), culture time (Time) and culture temperature (Temperature) were used for three-way ANOVA.

Table S3: Added C, Added N and Added P were used for three-way ANOVA.

Table S4: Biomarkers obtained from LEFSe analysis of the treatments.

Figure S1: The diversity of fine-root litter and different stages effects on soil CO_2 release rate.

Figure S2: The diversity of fine-root litter effects on soil properties.

Figure S3: Redundancy analysis of soil properties and microorganisms and correlation analysis of soil properties.

Figure S4: The diversity of fine-root litter effects on PLFAs of soil microorganisms.

Figure S5: Effects of fine-root litter moss loss on soil properties.

Figure S6: Effect of fine-root litter mass loss on soil microorganisms.

Figure S7: The diversity of fine-root litter effects on soil fungal alpha diversity.

Figure S8: The diversity of fine-root litter effects on relative abundance of soil fungi.

Figure S9: The diversity of fine-root litter effects on relative abundance of soil fungal trophic mode.

Figure S10: Cladogram of soil fungal in revegetation types via LEfSe method identifies the significantly different abundant taxa.

Data S1: Replication statement.

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