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

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Abstract

Many previous studies have focused on leaf litter decomposition in tropical ecosystems, but our understanding of the effect of root diversity on decomposition and soil respiration is still unclear. We investigated the decomposition of fine-roots from 21 dominant tree species from a tropic forest in a long-term, well-replicated incubation experiment with varying levels of root diversity. We measured fine-root mass loss and soil CO2 release and analyzed potential microbial drivers and related soil properties. Our results showed that as fine-root litter diversity increased, soil properties, microbial diversity, and fungal biomass changed nonlinearly, leading to the highest mass loss and soil CO2 release in the moderate diversity treatment group. Indirect effects of soil properties and microbial communities were larger than the direct effect of fine-root diversity. Our findings suggest that root diversity has a nonlinear effect on soil respiration during decomposition and emphasize the importance of protecting biodiversity.

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Authors' contributions

J.W. and D.C. designed the experiment and wrote the manuscript; D.C., S.X., S.L., S.Z., H.C., and X.D. conducted the fieldwork and laboratory work; J.W. and D.C. took part in statistical analysis. All authors have read and agreed to the published version of the manuscript.

Data accessibility statement

We confirm that all data supporting the results will be archived in the Dryad Digital Repository if the manuscript is accepted.

Abstract

Many previous studies have focused on leaf litter decomposition in tropical ecosystems, but our understanding of the effect of root diversity on decomposition and soil respiration is still unclear. We investigated the decomposition of fine-roots from 21 dominant tree species from a tropic forest in a long-term, well-replicated incubation experiment with varying levels of root diversity. We measured fine-root mass loss and soil CO_2 release and analyzed potential microbial drivers and related soil properties. Our results showed that as fine-root litter diversity increased, soil properties, microbial diversity, and fungal biomass changed nonlinearly, leading to the highest mass loss and soil CO_2 release in the moderate diversity treatment group. Indirect effects of soil properties and microbial communities were larger than the direct effect of fine-root diversity. Our findings suggest that root diversity has a nonlinear effect on soil respiration during decomposition and emphasize the importance of protecting biodiversity.

1 Introduction

Plant diversity is decreasing due to global climate change and human activity (Ohashi et al., 2019; Cao et al., 2022), with environmental pressures on plants expected to rise in the future as 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 plays a critical role in the global C cycle, 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). Despite many studies focused on aboveground litter decomposition, and estimating rates of root decomposition is challenging because roots are located underground, so that the effect of root diversity and root litter decomposition on soil C cycling has been frequently overlooked (Canessa et al., 2022).

Because root-derived C forms a larger portion of the relatively stable soil C pool than C originating in aboveground 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, root 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). The effects of mixed-quality litter on decomposition rate and nutrient release have been studied extensively (Man et al., 2020; Yang et al., 2022). Fine-roots (diameter < 2 mm) have higher nutrient content and are regarded as the most ephemeral in terrestrial ecosystems (McCormack et al., 2015), with the fine-roots of many plant species dying within in a year of their formation (Fogel, 1985). Fine-root biomass turnover accounts for c. 14-27% of net primary production (NPP) globally (McCormack et al., 2015) and is estimated to contribute 33% of annual litter inputs in forests around the world (Freschet et al., 2010). Labile fine-root litter inputs result in faster rates of decomposition, which in turn appear to activate microorganisms to stabilize soil organic matter (Cotrufo et al., 2013). Compared with production, growth, and death, the decomposition process of fine-roots has been studied less intensively (Smith et al., 2014; Man et al., 2020). Therefore, more accurate estimates of fine-root turnover are needed to better understand forest C dynamics (Le-Quéré et al., 2013). Tropical rainforests have high biodiversity, and plant roots in these forests intermingle and are decomposed in mixtures. Thus, a better understanding of root litter diversity and its effect on soil C cycling is imperative.

Previous investigations of the effect of root litter diversity on decomposition have inconsistent findings (Gripp et al., 2018). For example, in subtropical forests, litter diversity has positive effects, largely driven by mild to high temperatures (Liu et al., 2020). Microorganisms transfer nutrients between different litter types within mixtures, which can help to optimize 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). The mechanisms underlying the non-additive effects of diverse mixtures of leaf and root litter are still disputed, because experiments of different durations have yielded inconsistent results (*i.e.*, synergism or antagonism) (Lecerf et al., 2011). An increasing number of studies, however, suggests that litter species composition drives this effect and that composite litter chemistry may be the predominant factor under specific environmental conditions (Handa et al., 2014). Biological and abiotic factors, such as precipitation and temperature, should be central to modelled predictions of litter decomposition (Yang et al., 2022).

Microbial communities are the engines of decomposition, a fundamental process regulating ecosystem C cycling. Microbial decomposition converts C contained within detritus into CO_2 and releases nutrients for plant growth (Heijboer et al., 2018). Moreover, microbial ecologists consider fungi as the primary agents of decomposition (Glassman et al., 2018). Soil fungi play critical and unique roles in terrestrial ecosystem processes, and fungi are better equipped to decompose complex litter (Tedersoo et al., 2014). Most studies have focused on fine-root exudates, rhizosphere microorganisms, and fine-root function (Valverde-Barrantes, 2022). However, there are knowledge gaps about how the relationship between fungi and fine-root diversity influences decomposition in tropical rainforests.

To understand how fine-root diversity affects fine-root litter decomposition in tropical rainforests, we present a year-long laboratory experiment to elucidate the effects of two aspects of fine-root litter diversity on litter decomposition and soil CO_2 release. Since litter decomposition has an important effect on soil C cycling (McGuire & Treseder, 2010), soil CO_2 release and the mass loss of fine-root litter were measured to analyze how the soil C cycle responded to fine-root litter diversity. We hypothesized that: (1) The decomposition of mixed root litter has a non-additive effect, and the diversity of fine-root litter has a synergistic effect on fine-root litter mass loss and soil CO_2 release during decomposition; (2) The decomposition of fine-root litter and soil CO_2 release is nonlinearly affected by soil microbial biomass and fungal community structure.

2 Materials and methods

2.1 Materials

In October 2018, to collect fine-root litter (FRL) from 21 dominant tree species in a tropical rainforest in Xishuangbanna, Yunnan Province, China and surface soil (0-20 cm) was collected and transported to the laboratory in October 2020. Five sampling locations were randomly chosen. Humus on the soil surface was removed, sampled soil was mixed into a single composite sample, residual roots and stones were manually removed, and the soil was passed through a 2 mm sieve. Fine-roots (four replicates) were obtained from four randomly-selected trees from each totaling 12 families and 17 genera (Table 1). FRL was air-dried and sterilized with humid heat (121°C, 30 minutes, two successive sterilization treatments).

2.2 Experimental design

This study was a long-term (360 days), well-replicated incubation decomposition experiment. Nine treatments were investigated, with 0, 1, 3, 6, 9, 12, 15, 18, and 21 species (CK, M1, M3, M6, M9, M12, M15, M18, and M21 respectively) with 21 replicates in each group. 100 g of dry soil and 3 g of FRL were added to each 500 mL culture bottle for a total of 189 bottles. Soil moisture was held at 60% and culture bottles were incubated in the dark for 120 days at 25°C, 21.5°C and then 18°C for a total incubation period of 360 days. Soil CO_2 release was measured every month and residual FRL was recovered at the end of the experiment.

2.4 Sample measurements

Nutrient elements were measured in FRL and soil of 21 plants prior to the beginning of the experiment. After 12 months of culturing, 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 ℃ for fungal community and soil microbial phospholipid fatty acid (PLFA) analysis. Samples were used to determine physical and chemical properties of the soil, including soil organic carbon (SOC), dissolved organic carbon (DOC), total nitrogen (TN), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP), available phosphorus (AP), and pH.

Fresh soil was used to determine soil microbial PLFA and fungal community composition. Soil bacterial and fungal biomass was characterized by PLFA analysis (Chen et al., 2022). Fungal communities were profiled by sequencing amplicons targeting the fungal ITS sequence of the 16S 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 2 hours. A syringe was used to collect and transfer gas to 12 mL exetainers and CO_2 concentration was measured using a gas chromatograph (GC-2014, Shimadzu, Japan).

2.5 Calculations and statistical analysis

We used linear regression to examine generalized 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 of each soil CO_2 release rate and litter mass loss were calculated. The expected values and litter mixture were calculated by Wardle et al. (1997). We used a piecewise structural equation model (SEM) (Lefcheck, 2016) to assess the effects of FRL diversity on the biomass, diversity, and composition of fungal communities via changes in soil abiotic variables. Differences in soil properties, CO_2 release, microbial PLFAs, fungal alpha diversity, and fungal tropical mode relative abundance were compared using ANOVA and least significant difference (LSD) methods. All statistical analyses were performed using 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

FRL mass loss was nonlinearly correlated with FRL diversity (Figure 1A). The experiment was over, M9 had the maximum and M3 the minimum of FRL mass losses (Table S1). There was a nonlinear correlation between soil CO_2 release and FRL diversity (Figure 1, 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 effects of FRL diversity on soil properties were varied (Figure 2, 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). FRL mass loss 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).

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 (Figure S5-6). 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 index values of soil fungi (Figure S7). Bray-Curtis dissimilarities of soil fungi were significantly correlated with TN and pH (Figure S3B). Increased FRL diversity enhanced the relative abundance of Acomycota and decreased the relative abundance of Basidiomycota at the phylum level, increased the relative abundance of Sordariomycetes and decreased the relative abundance of Tremellomycetes at the class level; and increased the relative abundance of Chaetosphaeriales and Sordiales but decreased relative abundance of Tremellales at the order level (Figure S8, 9). Increased FRL diversity had no significant effect on saprotrophic fungi, but reduced Pathotroph-Saprotrph-Symbiotroph and Pathotroph-Symbiotroph fungi (Table S4, Figure S8-10).

3.3 Linking FRL mixed decomposition with soil properties

The TC, TN, and TP content of FRL was 335-440 g kg^{-1}, 4.85-16.18 g kg^{-1}, and 0.02-0.25 kg^{-1}, respectively (Table 1). Three-way ANOVAs showed 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 added C content of FRL (Table S3).

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 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

We investigated decomposition along a FRL diversity gradient and found that FRL diversity had positive, non-additive effects on mass loss and soil CO_2 release, and moderately diverse FRL mixtures had larger synergistic effects on FRL mass loss and soil CO_2 release. The mixture effects of M9 and M12 were larger than those of other treatments, consistent with our first hypothesis. Not only were FRL mixture effects related to FRL diversity, but they were also related to soil properties and FRL traits. Our results indicated that soil microbial biomass and fungal community structure were nonlinearly correlated with FRL diversity, supporting our second hypothesis. Collectively, our study indicated that diversity positively affects FRL decomposition, with moderate FRL diversity increases microbial activity and leads to accelerated decomposition, enhancing soil C cycling in tropical rainforests.

4.1 Effects of FRL diversity on the mass loss

Using a variety of FRL mixtures collected from a tropical forest, we found that FRL mass loss showed a non-additive effect and moderate FRL diversity had a larger positive effect on mass loss (Figure 1, 5). Consistent with our findings, a study has shown that litter diversity influences decomposition with non-additive effects (Liu et al., 2020). Globally, litter decomposition rates depend on climate, legacy of plant functional traits such as litter quality (Cornwell et al., 2008), and soil properties (Wan et al., 2022). For example, previous work has shown that the decomposition rate of root litter depends on C form (Chapin et al., 2011) and is not related to the concentration of N in soil and roots (Chen et al., 2002; Kaiser et al., 2015). However, our results showed that SOC, TN, NH_4^+-N, and NO_3^--N were positively correlated with FRL mass loss (Figure S5), which suggests that not only C, but also N, is an important driver of litter decomposition. Recent work found that litter input and decomposition can increase P availability (Wu et al., 2019), the opposite of our finding that FRL mass loss was negatively correlated with soil TP, AP, and pH. This is likely because of the low P content in tropical rainforest soil (Kochian, 2012) and the progressive P depletion resulting from microbial uptake and subsequent immobilization (Liang et al., 2020). Consistent with previous studies, we found that leaf litter accumulation reduces soil pH (Vitkova et al., 2015). This can be attributed to the increased decomposition of FRL, which drives higher rates of N mineralization and nitrification. These processes generate protons, ultimately causing a reduction in soil pH (Mueller et al., 2012).

We found that FRL diversity was significantly correlated with both microbial biomass and fungal diversity and moderate FRL diversity led to the maximum fungal diversity (Figure 3, S4). Plant diversity directly and indirectly affects litter decomposition (Sheng et al., 2019), and mixed litter likely contributes to mass loss because it provides richer nutrients for microbes (Lecerf et al., 2011; Gessner et al., 2010). Consistent with our findings, 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). However, high diversity can also increase substances that are difficult to decompose and inhibit microbial activity (Prieto et al., 2016; Man et al., 2020). Our work suggests that moderate FRL diversity can create positive feedback between mass loss and fungal activity, and may explain why the most diverse FRL was not associated with the highest mass loss. As more types of litter are added to a system, the concentration of hard-to-decompose substances increases, weakening the synergistic effect (Man et al., 2020). Also, some studies indicate that root decomposition is negatively affected by species richness and can inhibit mass loss in systems dominated by the decomposition of mixed roots (Prieto et al., 2017). FRL addition had no significant effect on the trophic mode of saprotrophic fungi, but it increased their relative abundance. Dominant fungi were mainly rhizosphere microorganisms. Variation in FRL diversity led to differences in decomposition, which in turn changed fungal community composition and trophic mode, *i.e.*, M9 treatment increased the abundance of fungi belonging to the Sordariales order, which can stimulate organic matter decomposition (Zhou et al., 2021).

Our study found that moderate FRL diversity had the largest synergistic effect and resulted in the greatest mass loss. 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 the mass loss of litter mixtures or individual components (Gessner et al., 2010). Previous work has shown that effects of mixed litter are related to decomposition time and plant characteristics (Osono et al., 2006). For example, the decomposition rate of FRL varies with plant type: in general, FRL from broad-leaved species is higher than FRL from coniferous species. Moreover, higher diversity in forested systems will lead to antagonistic effects in litter decomposition (Silver & Miya, 2001). However, some studies have found that the decomposition of community roots is negatively affected by species richness, which inhibits mass loss in root mixtures compared with the respective single species (Prieto et al., 2017; Man et al., 2020). These inconsistencies indicate the need for additional research.

4.2 Effects of FRL diversity on soil CO_2 release

We found that FRL addition promoted soil CO_2 release, and moderate FRL diversity was associated with higher soil CO_2 release (Figure 1, 5). Litter diversity has a significant effect on litter decomposition (Zhou et al., 2020), and previous work has shown that litter traits are the ultimate cause of non-additive synergistic or antagonistic effects (Chen et al., 2018). In this study, no antagonistic effects were observed in mixed FRL, probably because the leaching and transfer of nutrients and inhibitory compounds between litter species can result in synergistic litter mixing effects (Handa et al., 2014). The non-additive antagonistic effect on cumulative soil CO_2 release in root 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 content increases antagonism, influencing cumulative soil CO_2 (Gessner et al., 2010; Man et al., 2020). A recent study suggested that high species diversity of root litter reduces the cumulative release of soil CO_2 (Man et al., 2020), but this is not consistent with our findings, likely because of differences in litter properties and ecosystem type (Cornwell et al., 2008; Smith et al., 2014).

We found that the moderate FRL diversity drove the highest CO_2 release rate because of its high mass loss and microbial diversity. This may be due to the different nutrients provided by the decomposition of different FRLs (Heijboer et al., 2018), which provide adequate substrate for soil microbial respiration, thereby enhancing soil CO_2 release. Similarly, fungi can promote nutrient release from litter, enhancing soil nutrient availability (Tan et al. 2021). Moreover, fungi can improve soil permeability (Liu et al., 2019). All these factors can promote microbial activity and promote soil respiration. Litter removal reduces the diversity of fungi, especially saprotrophic fungi, because it is more capable of decomposing soil carbon than other fungi (Zhou et al., 2021). This is consistent with our findings that the M9 treatment was associated with a relatively high abundance of saprophytic fungi. Other work has found a close correlation between increases in soil respiration and increases in total PLFAs (Wu et al., 2017), demonstrating that fungal and bacterial biomass has a significant effect on soil respiration (Figure 4A). A previous study has also shown that a decrease in NH_4^+-N stimulates microbial activity, which enhances soil respiration (Veresoglou et al., 2012). However, NH_4^+-N content in moderate diversity FRL was higher in our study because autotrophic respiration responds more strongly to nutrient inputs than heterotrophic respiration. In future research on the effect of fine-roots on soil C cycling, fine-root respiration should be given adequate attention.

5 Conclusions

The results of our long-term incubation experiment showed that there was a nonlinear correlation between FRL diversity and mass loss and soil CO_2 release, with moderate diversity FRL having the greatest effect. Differences root diversity resulting in different releases of nutrients, with moderate FRL diversity had the largest effect. FRL decomposition changed soil microbial biomass and fungal community composition. The resulting changes in soil physical and chemical properties and microbial community composition directly and indirectly affected mass loss and soil CO_2 release, and the effects of soil properties on both were higher than that of microorganisms. In addition, added FRL altered fungal trophic mode, and moderate FRL diversity resulted in higher relative abundance of saprophytic fungi. Our findings suggest that root diversity has a nonlinear effect on soil respiration during decomposition, and it is thus imperative to protect biodiversity in tropical forests.

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Conflict of interest

All authors declare that there is no conflict of interest.

Data Availability Statement

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

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Tables legends

TABLE 1. Classification of 21 tropical tree species and their fine-root nutrient contents. AM, arbuscular mycorrhiza; EM, ectotrophic mycorrhiza; TC, total carbon; TN, total nitrogen; TP, total phosphorus.

Figure legends

FIGURE 1. The relationship between fine-root litter diversity, mass loss, and soil CO_2 release, and the effect of soil stoichiometry on soil CO_2 release rate. Dotted line indicates non-significance (P>0.05). Soil CO_2 release rate (RR) $=M/Vm \times 460\ (C2\text{-}C1) \times 1/1000m \times P/P0 \times T0/T$. 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}); Vm is the molar volume of 22.4 (L mol^{-1}); 460 is the gas volume to be measured in the culture bottle (mL); $C1$ is the concentration of the gas to be measured (ppm) in the container; $C2$ 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; $P0$ is standard atmospheric pressure of 101.325 kpa; $T0$ is absolute temperature under standard conditions of 273.15 K; T is the absolute experimental temperature (273.15+t (°C)) K. Litter mass loss (ML)=($m0$-$m1$)/$m0$×100%. Where $m0$ is the initial FRL dry weight and $m1$ is the dry weight of FRL at the end of the experimental period.

FIGURE 2. Effects of fine root litter diversity on soil properties. Dotted line indicates non-significance (P>0.05).

FIGURE 3. Effects of fine root litter diversity on soil microorganisms. Dotted line indicates non-significance (*P*>0.05).

FIGURE 4. Mixture effects on the decomposition of fine root litter. *O*: observed values, *E*: expected values. Asterisks indicate significant deviations from zero (Student's t tests; *<0.05, **<0.01, ***<0.001). 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* of 21 samples. Different lowercase letters indicate significant differences at $P < 0.05$ between treatments. Based on the values obtained from single species litter, we calculated the expected values (*E*) $= \sum_{i=1}^{S} R_i / S$. 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-test 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). Litter mixture effect = $(O - E)/E$.

FIGURE 5. Piecewise structural equation model (SEM) of the effect of fine root litter diversity on soil CO_2 release and fine root litter mass loss. C and D depict the direct, indirect, and total effect values of each dependent variable. SOC: soil organic carbon, DOC: soil dissolved organic carbon, TN: soil total nitrogen, NH_4^+-N: ammonia nitrogen, NO_3^--N: nitrate nitrogen, TP: total

phosphorus, AP: available phosphorus, C:N: ratio of SOC to TN, C:P: ratio of SOC to TP, N:P:, ratio of TN to TP, C:N:P: ratio of SOC, TN and TP. Microbial diversity includes bacterial and fungal Shannon index and pairwise mean Bray-Curtis dissimilarity. Values associated with arrows represent standardized path coefficients. Values associated with response variables indicate the proportion of variation explained by relationships with other variables.

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*	*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

TABLE 1

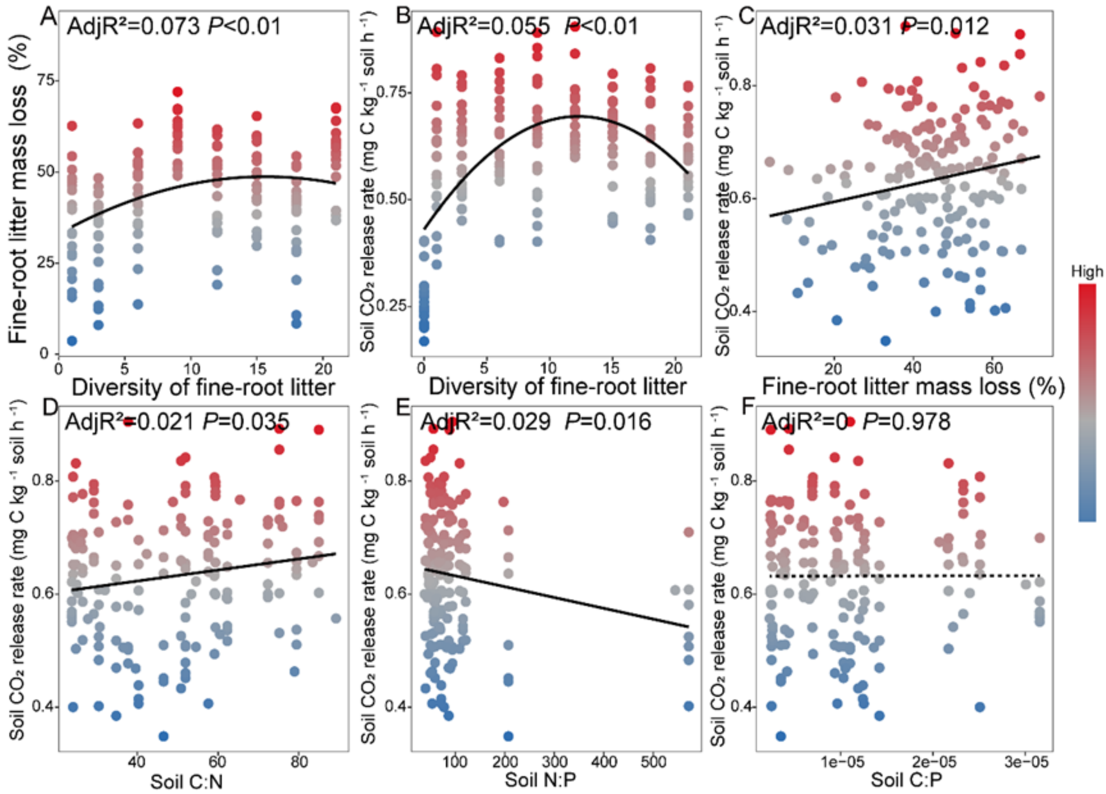
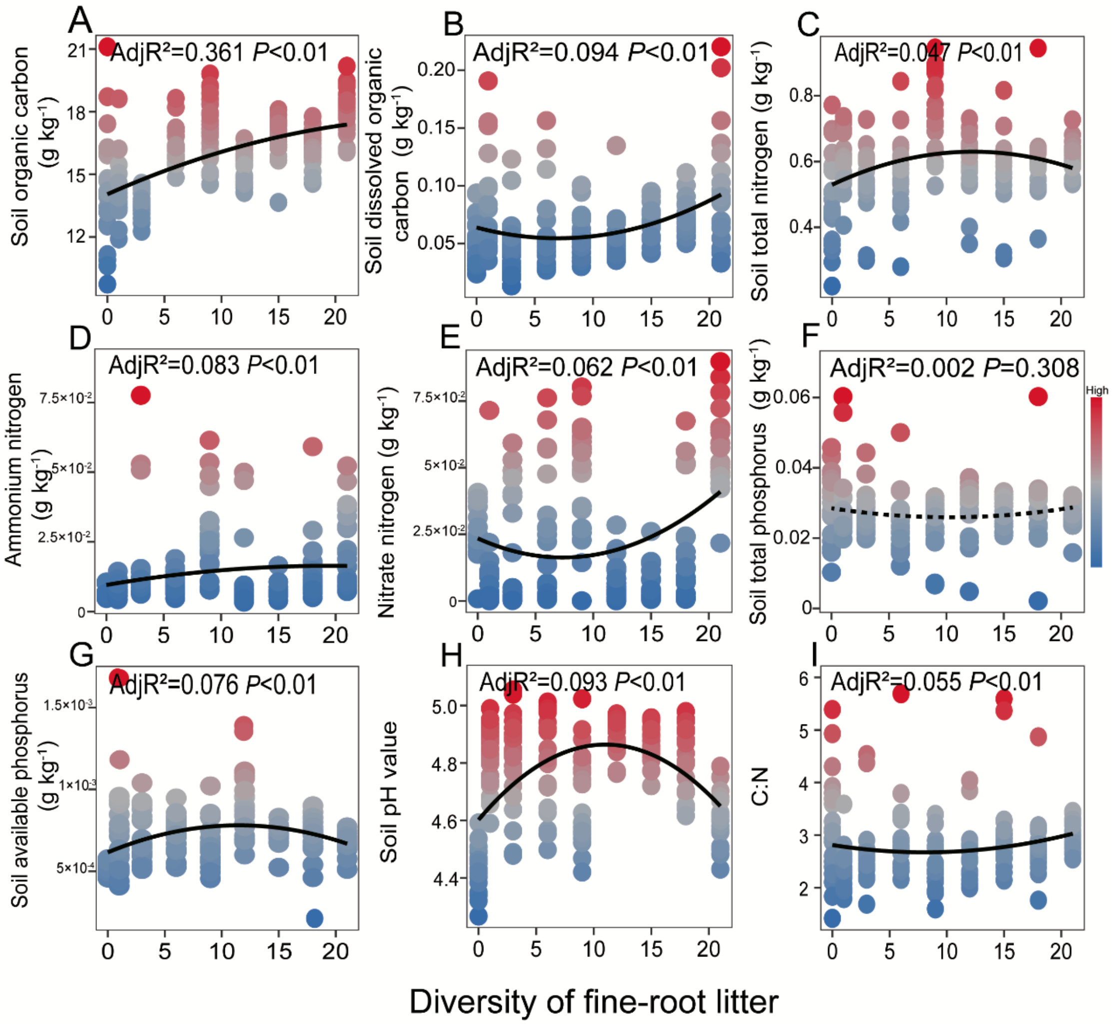

A
AdjR²=0.073 P<0.01
Fine-root litter mass loss (%)
Diversity of fine-root litter
B
AdjR²=0.055 P<0.01
Soil CO₂ release rate (mg C kg⁻¹ soil h⁻¹)
Diversity of fine-root litter
C
AdjR²=0.031 P=0.012
Soil CO₂ release rate (mg C kg⁻¹ soil h⁻¹)
Fine-root litter mass loss (%)
High
D
AdjR²=0.021 P=0.035
Soil CO₂ release rate (mg C kg⁻¹ soil h⁻¹)
Soil C:N
E
AdjR²=0.029 P=0.016
Soil CO₂ release rate (mg C kg⁻¹ soil h⁻¹)
Soil N:P
F
AdjR²=0 P=0.978
Soil CO₂ release rate (mg C kg⁻¹ soil h⁻¹)
Soil C:P

FIGURE 1



570

571 **FIGURE 2**

572

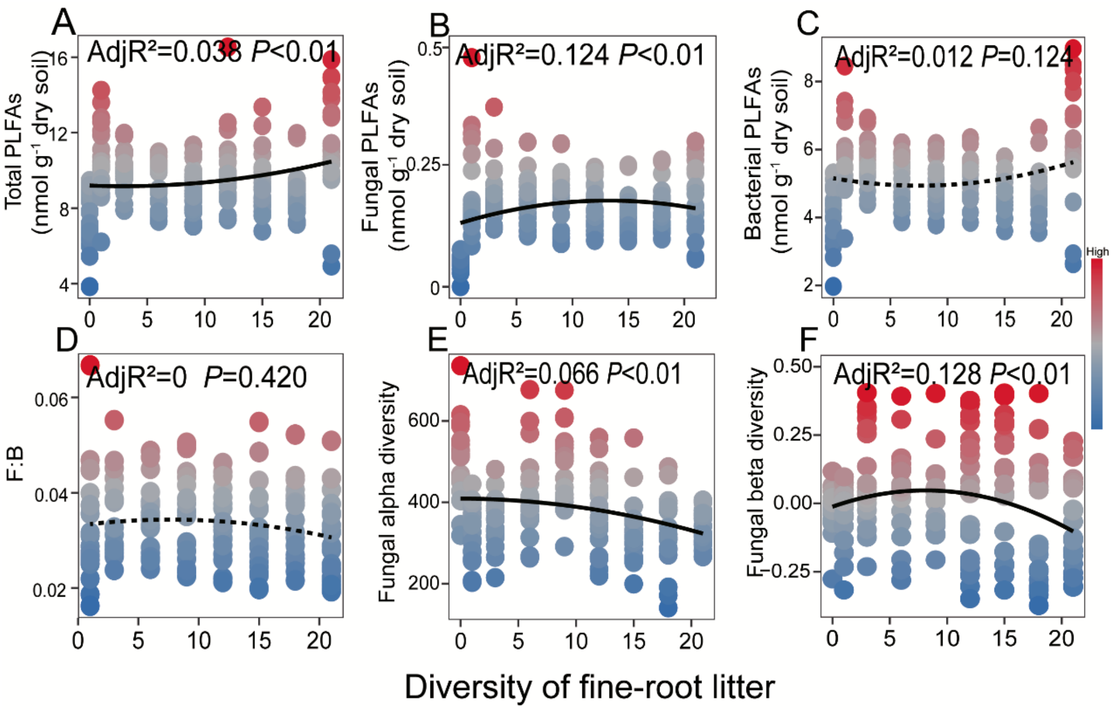
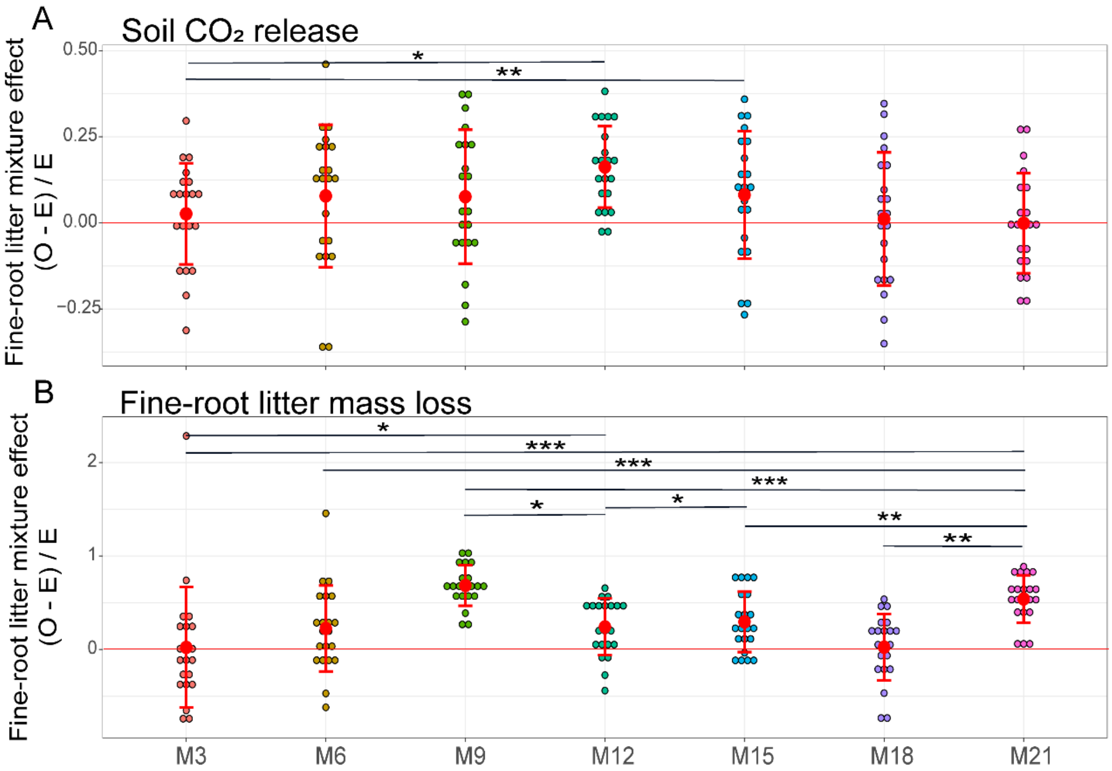

A
AdjR²=0.038 P<0.01
Total PLFAs (nmol g⁻¹ dry soil)
B
AdjR²=0.124 P<0.01
Fungal PLFAs (nmol g⁻¹ dry soil)
C
AdjR²=0.012 P=0.124
Bacterial PLFAs (nmol g⁻¹ dry soil)
High
D
AdjR²=0 P=0.420
F:B
E
AdjR²=0.066 P<0.01
Fungal alpha diversity
F
AdjR²=0.128 P<0.01
Fungal beta diversity
Diversity of fine-root litter

FIGURE 3



A
Soil CO₂ release
Fine-root litter mixture effect
(O - E) / E
0.50
0.25
0.00
−0.25
*
**
B
Fine-root litter mass loss
2
1
0
*
***
***
***
*
*
**
**
M3
M6
M9
M12
M15
M18
M21

FIGURE 4

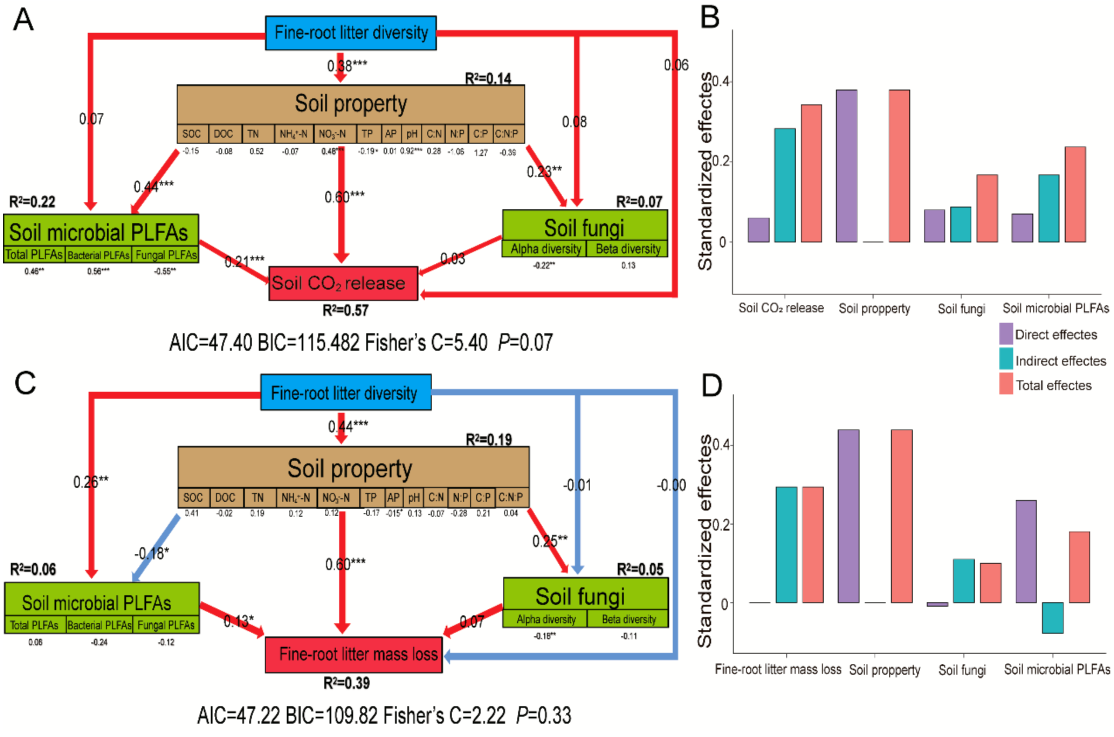

A
Fine-root litter diversity
Soil property
R²=0.14
Soil microbial PLFAs
Total PLFAs
Bacterial PLFAs
Fungal PLFAs
R²=0.22
Soil fungi
Alpha diversity
Beta diversity
R²=0.07
Soil CO₂ release
R²=0.57
AIC=47.40 BIC=115.482 Fisher's C=5.40 P=0.07
B
Standardized effectes
Soil CO₂ release
Soil propperty
Soil fungi
Soil microbial PLFAs
Direct effectes
Indirect effectes
Total effectes
C
Fine-root litter diversity
Soil property
R²=0.19
Soil microbial PLFAs
R²=0.06
Soil fungi
R²=0.05
Fine-root litter mass loss
R²=0.39
AIC=47.22 BIC=109.82 Fisher's C=2.22 P=0.33
D
Standardized effectes
Fine-root litter mass loss
Soil propperty
Soil fungi
Soil microbial PLFAs

FIGURE 5