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Negative effects of litter richness on root decomposition in the presence of detritivores

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Summary

1. Decomposition is a vital process underlying many ecosystem functions. Although a growing number of studies have tested how litter richness affects the decomposition of aboveground plant organs, knowledge remains limited about the decomposition of root mixtures. Here, we used a field experiment in a subtropical forest to investigate how species richness in root litter mixtures (air-dried fresh fine roots) affects the decomposition of root litter material.
2. Based on the concept of resource complementarity, we hypothesized that root litter would decompose faster as the richness of the root litter mixture increased. In addition, we expected the presence of detritivores to modify the effect of root richness on mass loss, because detritivores might experience bottom-up effects from specific plant species and might affect microbial decomposer communities.
3. We found that the richness level of root litter mixtures did not affect mass loss in the absence of detritivores. In the presence of detritivores, all root litter types decomposed faster. Notably, the positive effect of detritivores was stronger at low root litter richness than at high root litter richness, particularly during the early stages of decomposition (the first two sampling points) when litter mass loss was roughly double at low root litter richness compared to that at high root litter richness. The composition of the root fungal community

measured at the last sampling point did not differ significantly across root richness levels, and was not affected by the presence of detritivores.

4. *Synthesis.* Our findings demonstrate that detritivores modify the relationship between root litter diversity and root litter decomposition in subtropical forest ecosystems. This highlights an importance of cascade effects between different trophic organisms on ecosystem functioning.

Key-words: Litterbags, Mass loss, Microbial decomposer, Plant species richness, Roots, Subtropical forest

Introduction

Over the last decade, an increasing number of studies have aimed to unravel the role of roots and root traits in the functioning of ecosystems (Adam *et al.* 2006; Hobbie *et al.* 2010; Li *et al.* 2015; Guerrero-Ramírez *et al.* 2016). As a result, it has been acknowledged that root traits and root diversity have important consequences on ecosystem functioning (Bardgett, Mommer & de Vries 2014). However, knowledge on root decomposition processes remains scarce, and most of our understanding of litter breakdown is still based on the decomposition of aboveground plant organs and processes. Yet, roots form a major source of litter in the soils and might be key drivers of soil carbon and nutrient cycling (Parton *et al.* 2007; Freschet *et al.* 2013). To date, studies that have aimed to understand how litter is broken down have focused on litter from single plant species. However, as plant species often grow in diverse mixtures, it is essential to test how root litter richness drives the decomposition of mixed-species litter (Gartner & Cardon 2004; Gessner *et al.* 2010). Here, we investigate how changes in root litter richness affect the decomposition of root material.

The impact of litter mixing has been studied intensively with respect to leaf litter decomposition. Positive effects of leaf litter mixing on decomposition have been attributed to the niche complementarity of decomposers in terms of resource acquisition (Cardinale *et al.* 2007; Gessner *et al.* 2010). Mixed leaf litters supply a high diversity of resources for detritivores and optimize nutrient acquisition for microbial decomposers (Eisenhauer 2012; Chapman *et al.* 2013), thus, enhancing the decomposition of mixed litter (Loreau 2001). Moreover, in mixed leaf litter, the functional diversity of detritivores and microbial decomposers might increase, which could stimulate the overall decomposition of leaf litter mixtures (Heemsbergen *et al.* 2004; Ebeling *et al.* 2014; Handa *et al.* 2014). In addition, fungi-driven nutrient transfer among mixed leaf litters decreases nutrient limitation for some constituent species and speeds up the overall decomposition of leaf litter mixtures (Hättenschwiler, Tiunov & Scheu 2005; Schimel & Hättenschwiler 2007). Finally, the mixing of multiple plant species with dissimilar trait spectrums enhances habitat structure and heterogeneity (Hättenschwiler, Tiunov & Scheu 2005), benefiting different decomposer organisms and, thus, enhancing decomposition (Wardle *et al.* 2003). However, knowledge remains limited about whether litter mixing also has a positive effect on plant roots.

In addition to the effects of mixing diverse species, specific plant functional groups or species in a mixture might have strong effects. For example, the “mass ratio hypothesis” predicts that dominant plant species strongly influence ecosystem functioning (Grime 1998; Smith & Knapp 2003), including decomposition (Cárdenas *et al.* 2017). In the case of decomposition, plant species with specific functional traits are key drivers of litter breakdown, because they have a strong impact on microbial decomposers and detritivores (Wardle, Bonner & Nicholson 1997; Milcu *et al.* 2008; Treplin, Pennings & Zimmer 2013). Moreover, detritivores and microbial decomposers selectively use heterogeneous resources supplied by different constituent plant species within leaf litter mixtures (Heemsbergen *et al.* 2004;

Hättenschwiler & Gasser 2005; Mukhopadhyay, Roy & Joy 2014). Detritivores and microbial decomposers preferentially utilize nutrient-rich leaf litter to maximize energy intake and optimize the nutrient balance of their tissues (Treplin, Pennings & Zimmer 2013), while avoiding plant species with inhibiting compounds, such as tannins (Coq *et al.* 2010). This selective utilization of specific species in leaf litter mixtures leads to selection effects (Loreau & Hector 2001) in the decomposition of mixed leaf litter. The presence of certain decomposers might even change how leaf litter richness affects litter mass loss (Eisenhauer, Reich & Isbell 2012). Although, there is some evidence that the root litter quality of particular species is more important at driving decomposition in mixtures than species richness effects (Guerrero-Ramírez *et al.* 2016), it remains unclear to what extent constituent plant species affect the breakdown of root litter mixtures.

The consequences of litter mixing on decomposition depend on the interaction between decomposers and constituent plant species within leaves (Wickings *et al.* 2012) and root litter mixtures (Fujii & Takeda 2012), in addition to interactions between detritivores and microbial decomposers (Hättenschwiler, Tiunov & Scheu 2005; Gessner *et al.* 2010; Mukhopadhyay, Roy & Joy 2014). Detritivores drive the performance, metabolic capacities, and composition of microbial communities by changing habitat conditions (Johnson & Rasmann 2015) and litter physicochemical characteristics (Mukhopadhyay, Roy & Joy 2014), as well as by directly grazing on fungi and disrupting mycelial networks (Lussenhop 1992). In turn, the microbial conditioning of litter might affect the feeding activity of detritivores *via* altering litter chemical composition during decomposition processes (Wickings *et al.* 2012; Harrop-Archibald *et al.* 2016), and function by producing cues for detritivores to detect fresh and nutrient-rich resource patches (Bonkowski, Griffiths & Ritz 2000). Therefore, it is important to understand how interactions between detritivores and microbial decomposers affect the decomposition of mixed root litter.

In this study, we investigated root litter effects on its decomposition rates. We also aimed to elucidate how root litter quality, the decomposing environment (i.e. soil moisture and pH), detritivores, and microbial decomposers affect the decomposition process. We specifically tested three hypotheses. First, we hypothesized that root litter with mixed species would decompose faster than monospecific root litter, because of complementary resource supply for decomposers (Chapman *et al.* 2013; Handa *et al.* 2014), particularly during the early stages of decomposition. The effect of mixed litter might decrease over time because the composition of litter compounds might converge, lowering resource complementarity (Coleman *et al.*, 2004; Wallenstein *et al.* 2013; Berg & McClaugherty 2014). Moreover, fresh root materials that are rich in nutrients might contribute to a stronger positive effect on root richness during the early stages of decomposition because higher-quality litter might accelerate the breakdown of recalcitrant litter species in a mixture (Hättenschwiler & Gasser 2005). Second, we hypothesized that macro-detritivores would stimulate root mass loss due to the ingestion and fragmentation of litter (Mukhopadhyay, Roy & Joy 2014), and that macro-detritivores would enhance the effects of root litter richness on decomposition. This is because litter mixtures with higher species richness might be of higher quality making them more attractive to litter-feeding macro-detritivores (Hättenschwiler & Gasser 2005). Third, we expected that the diversity of microbial decomposers would increase with root litter richness, contributing to the positive effects of root richness on mass loss (Loreau 2001), with microbial-mediated decomposition being suppressed by detritivores because of their feeding on specific microbes (Gessner *et al.* 2010). To test our hypotheses, we set up a three-year field experiment in a subtropical forest in southern China where we decomposed fresh fine root litter mixtures (root diameter less than 2 mm) in litter bags that were accessible or not accessible to detritivores. Litter bags were harvested to determine litter mass loss, at eight time points. In addition we measured soil microbial biomass and soil physicochemical

parameters at each harvest. At the end of the experiment, we also determined fungal community composition. Our results are expected to provide new insights into the importance of detritivores in decomposing root litter in systems containing different combinations of plants.

Material and methods

STUDY AREA

This study was conducted in Gutianshan (GTS) National Nature Reserve (29°8'18" - 29°17'29" N, 118°2'14" - 118°11'12" E; Bruelheide *et al.* 2011), which is located in Kaihua County of Zhejiang Province in southern China. The GTS reserve covers approximately 8,107 ha of subtropical forest. The main vegetation is subtropical evergreen broad-leaved forest dominated by *Castanopsis eyrei* and *Schima superba* (Yu *et al.* 2001). The mean annual temperature is 15.3 °C, with the highest and lowest mean monthly temperature occurring in July (28.9°C) and January (4.1 °C), respectively (Zhu *et al.* 2013). Mean annual precipitation is 1,964 mm, with the wet season occurring from March to June and in September, while the dry season occurs from July to August and from October to February. Topography is rugged, with steeply sloping mountains in the GTS reserve, and the soil is classified as Ultisol according to US soil taxonomy (Zhang *et al.* 2011).

EXPERIMENTAL DESIGN

In 2010, we established a 3-year litterbag study in the GTS subtropical forest to test our hypotheses. We used the fresh fine roots of eight plant species. Four canopy tree species commonly coexist in the natural subtropical forests of this region, including *Castanopsis eyrei* (Champ. ex Benth.) Tutch., *Schima superba* Gardn. et Champ., *Daphniphyllum oldhamii* (Hemsley) K. Rosenthal, and *Pinus massoniana* Lamb. (with approximately 43.8%

percentage co-occurrence of these four canopy tree species). In addition, we used two understory tree species (i.e. *Syzygium buxifolium* Hook. et Arn and *Rhododendron ovatum* Planch.) and two shrubs (i.e. *Rhododendron simsii* Planch. and *Eurya rubiginosa* H. T. Chang var. *attenuate* H. T. Chang) that commonly grow under the four canopy tree species.

The roots of the eight plant species were used to create root litter mixtures with four richness levels (1, 2, 4, and 8 species) (Fig. S1, left panel). Fresh fine roots (root diameter less than 2 mm) were collected from a seedling garden adjacent to our experimental field, where all of the plant seedlings that we used in our experiment had grown on homogeneous soils and environmental conditions for two years. To start with standardized litter mixtures, we sampled fresh fine roots because, to date, it is not possible to identify the stage of root senescence in decomposing roots; yet, this variable could strongly influence litter decomposition (Hobbie *et al.* 2010). The fine roots were air-dried and used to fill the nylon litterbags (10 cm × 15 cm with a 50 µm mesh). We created root litter mixtures according to a random partitions design following a broken stick procedure. In brief, eight plant species were randomly partitioned into two groups with four plant species, and then four plant species within each group were separately distributed into two groups again. The partitioning process was continued, with each partition producing a mixture of two plant species. As a result of the partitioning procedure, root litter mixtures of the same richness level were composed of different plant species, thus, reducing sampling effects (Fig. S1; Wardle 1999).

We filled litterbags with 6 g of fine roots. For root litter mixtures, the root amounts were equivalent for each plant species in the mixture. In total, there were 15 different types of litterbags; namely, eight types of monospecific root litters, four different two-species mixtures, two different four-species mixtures, and one eight-species mixture. The litterbags were buried in the field, each at the same depth of 0-15 cm on 11 November, 2010. We carefully made an incision in the soil at a 45° angle with a shovel. Then, we put the litterbags

into these soil incisions, and kept the top of these litterbags at about 0 cm depth and the bottom of them near at about 15 cm depth (Fig. S1, right panel). We randomly selected three 30×30 m areas with enough distance (geographic coordinates of these blocks: $29^{\circ} 14' 59''\text{N}/118^{\circ} 7' 13''\text{E}$, $29^{\circ} 14' 49''\text{N}/ 118^{\circ} 6' 44''\text{E}$, and $29^{\circ} 14' 33''\text{N}/ 118^{\circ} 5' 59''\text{E}$, respectively) in the GTS subtropical forest as blocks. Each 30×30 m area was divided into nine 10×10 m quadrats, of which the four corners of each block were used for the placement of the litterbags. Given the steep slope of the three blocks, where average slope was 34%, 26%, and 32%, respectively, each quadrat was assigned as a replicate litterbag deployment. The subset of litterbags was deployed at the centre of each 10×10 m quadrat, where the 15 different types of litterbags were randomly arranged.

During the experiment, some of the litterbags were invaded by unidentified macro-detritivores. In the studied subtropical forest, xylobiont arthropods are highly abundant and might contribute to root litter decomposition (Luo *et al.* 2011). The invaded litterbags were identified by small holes in the mesh (for the percentage of litterbags with holes, see Table 1 and Fig. S2). As the macro-detritivores accessed part of the litterbags of all root richness levels, we used their presence as a treatment factor to investigate how detritivore activity altered root mass loss.

Litterbags were retrieved at eight samplings times; namely, January (97 d), April (170 d), August (278 d), and October (357 d) 2011, February (482 d) and October (737 d) 2012, and January (826 d) and April (921 d) 2013. The total number of litterbags was composed of $15 \text{ types} \times 3 \text{ blocks} \times 4 \text{ replications per block} \times 8 \text{ sampling times} = 1,440$ litterbags. At each sampling time, 180 litterbags were harvested ($15 \text{ types} \times 3 \text{ blocks} \times 4 \text{ replicates}$).

Decomposing roots collected each time were carefully removed from the litterbags. Soil stuck to the roots was removed using a soft bristle brush, and the cleaned roots per sample were weighed after being dried at 60°C for 48 h. Surface soils at 0–15 cm depth were

sampled at the same time when collecting litterbags, to estimate soil physicochemical and microbial parameters. We randomly collected three soil cores from the area where the litterbags were placed, and we combined them in one composite soil sample per plot. Thus, a total of 12 soil samples were obtained per sampling time.

ROOT AND SOIL PHYSICOCHEMICAL PROPERTIES

For each monospecific root litter type and each root mixture, six replicate root samples were collected from the initial root litter pool (i.e. collected from the seedling garden) to measure the chemical composition of the initial root litter before deploying root litter materials in the forest field. The initial root samples were dried at 40°C for 48 h to a constant weight, and were then ground to measure various root chemical properties, including carbon (C), nitrogen (N), phosphorus (P), calcium (Ca), potassium (K), and magnesium (Mg) content. Root C and N content was estimated using a CHNOS Elemental Analyzer (vario EL III, CHNOS Elemental Analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). Root P, Ca, K, and Mg contents were analysed using an Inductive Coupled Plasma Emission Spectrometer (ICAP 6300 ICP-OES Spectrometer, Thermo Scientific, USA).

At each time point when the litterbags were harvested, the fresh weight of the root litter was measured. Then, the root litter was dried at 40 °C for 48 h to a constant weight. After drying, we used the percentage difference between the fresh and dry weight of the root litter in each litterbag to calculate root mass loss. Surface soil samples were passed through a 2-mm sieve, and were used to measure soil abiotic properties. To determine soil moisture, we dried soil samples at 105 °C for 48 h. Soil pH was measured in a 1:2.5 soil to water ratio using a glass electrode (Thermo Orion T20, USA). Soil organic C and N concentrations were determined using a CHNOS Elemental Analyzer (vario EL III, CHNOS Elemental Analyzer, Elementar Analysensysteme GmbH, Germany).

MICROBIAL COMMUNITY

We used a subsample of each soil sample to determine the composition of the microbial community using phospholipid fatty acid (PLFA) profiles. We extracted, fractionated, and quantified PLFAs in the fresh soil samples according to the procedure of Bossio & Scow (1998). The procedure we used is described in detail in Zhang *et al.* (2013). We used the saturated PLFAs i15:0, a15:0, i16:0, a17:0, i17:0, 16:1 ω 7c, 18:1 ω 5c, cy17:0, and cy19:0 to calculate bacterial PLFA biomass (Frostegård & Bååth 1996; Zak *et al.* 2003). Three unsaturated PLFAs, i.e. 18:1 ω 9c, 18:2 ω 6c, and 18:3 ω 6, were used to determine the biomass of fungal PLFA (Zak *et al.* 1996 and 2003).

At the end of the experiment, we determined the OTU composition of the fungal communities in the root litter using the Illumina MiSeq platform (Schmidt *et al.* 2013). DNA isolation was performed using Plant Genomic DNA Kit (Tiangen Biotech Co., China). Amplification for fungal ribosomal Internal Transcribed Spacer 1 (ITS1) was carried out in a total volume of 20 μ l, including 10 ng template DNA, 4 μ l 5 $\times$ FastPfu Buffer, 2 μ l 2.5 mM dNTPs, 0.4 μ l FastPfu Polymerase, and 0.8 μ l 5 μ M primer 1723F (5'-barcode-CTTGGTCATTTAGAGGAAGTAA-3'), and 0.8 μ l 5 μ M primer 2043R (5'-GCTGCGTTCTTCATCGATGC-3'). PCR conditions were 2 min at 95 $^{\circ}$ C, followed by 25 cycles of 30 s at 95 $^{\circ}$ C, 40 s at 55 $^{\circ}$ C, and 50 s at 72 $^{\circ}$ C, and a final extension at 72 $^{\circ}$ C for 5 min. PCR products extracted from 2% agarose gels were purified by AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions, and were sequenced on an Illumina MiSeq platform following standard protocols. The obtained raw sequence data were demultiplexed and quality-filtered as described by Zhang *et al.* (2016). We finished picking OTU and taxonomy assignment, respectively, using the pick_open_reference_otus.py and assign_taxonomy.py scripts in

QIIME based on the ITS 12-11 dataset (QIIME http://qiime.org/home_static/dataFiles.html).

We removed singletons of the OTU table using `filter_otus_from_otu_table.py` script. The ITSx was used to remove plant sequences from the OTU table (Bengtsson- Palme *et al.* 2013). We rarified the OTU table using `single_rarefaction.py` script in QIIME at a sampling depth of 4,000 to eliminate sampling depth heterogeneity, and, thus, obtained the final OTU table for the statistical analysis of fungal community composition. The sequences that were obtained from the QIIME procedure were submitted to the GenBank of NCBI (MF789354 - MF794206).

STATISTICS

We used a mixed effect model from the `asreml` package (Gilmour *et al.* 2015) suited for slightly unbalanced data, with plot nested in the block as a random factor, while decomposing time, root litter richness level, detritivore presence, and their interactions were used as fixed factors to test their effects on root mass loss. We conducted a Tukey's HSD test in the `agricolae` package (de Mendiburu 2014) to evaluate differences among means.

In addition, we performed partial regression analyses, using root mass loss as the response variable to test the net effect of each of the explanatory variables; namely, detritivore presence, root richness, fungal and bacterial PLFA biomass, root C/N ratio, root P, root K, root Ca and Mg, soil moisture, and pH. Taking the explanatory variable of detritivore presence as an example, we first performed a mixed effect model with all of the stated explanatory variables, except detritivore presence, as fixed factors and plot nested in the block as a random factor. The residual from this model is a latent variable representing the part of the variance on root mass loss that all other tested variables (except for detritivores) could explain. Thus, the residual variance was used to test the net effect of detritivore presence on root mass loss by ordinary least squares (OLS) linear regression.

The OTU matrix of fungi living in root litter obtained was based on QIIME workflow script, and was used to provide more detailed information about the fungal communities in response to detritivores and root richness levels. Three-dimensional non-metric multidimensional scaling (3D-NMDS) was performed to analyse how the composition of the fungal community affected root litter. The first three axes of 3D-NMDS were used to test the relationship between the root litter-inhabiting fungal community and root mass loss *via* the *cor.test* function. The *envfit* function in *vegan* package (Jari *et al.* 2016) was used to test the associations of fungal communities on root litter with respect to detritivore presence, root richness, initial root nutrients, soil microbes, and environmental parameters. A bipartite network of root-inhabiting fungi phyla linking to focal plant species was produced by the *plotweb* function in the package *bipartite*. We performed a linear mixed effect model, with detritivore presence and root litter richness as fixed factors and plot nested in the block as a random factor, to test the effects of detritivore presence, root litter richness, and their interaction on the dominant OTUs (accounting for at least 1% of relative abundance). A heat map of these dominant fungal OTUs was drawn using the function *heatmap.2*. All data were analysed using R statistical software (R 3.3.2; R core team 2015)

Results

EFFECTS OF ROOT LITTER RICHNESS ON MASS LOSS

Root mass loss was not affected by the richness of the root mixtures (i.e. single, two, four, and eight plant species) in the litterbags without detritivores, but was higher in the single root litterbags compared to the litterbag mixtures with detritivores (Fig. 1 and Table 2). The richness effect of root mixtures on mass loss in the litterbags with detritivores was consistent over time, as indicated by the non-significant three-way interaction between detritivores $\times$ richness $\times$ decomposing time (Table 2).

TEMPORAL FLUCTUATIONS IN ROOT LITTER MASS LOSS

The mass loss of the root litter showed significant temporal dynamics (Fig. 2 and Table 2).

The effect of the presence of detritivores significantly interacted with time (Fig. 2 and Table 2). Specifically, the presence of detritivores caused a greater increase in root mass loss at the earlier sampling points compared to the later sampling points (Fig. 2). We combined the mass loss data of all richness treatments to calculate the means of mass loss with and without detritivores per sampling time. At the first three sampling times (97, 170, and 278 d), we found that detritivores enhanced root mass loss by 160%, 95.3%, and 128%, respectively. In comparison, at the last five sampling times, root mass loss was only enhanced by 42.1%, 74.9%, 26.8%, 38.3%, and 63.4%, respectively (Fig. 2).

EFFECTS OF DETRITIVORES ON ROOT LITTER MASS LOSS

Overall, the mass loss of the root mixtures was significantly faster when detritivores accessed the litterbags (Figs. 1 and 2). Moreover, detritivore presence caused a negative root richness effect on root litter decomposition (see first paragraph of the Results; Fig. 1 and Table 2). The percentage of litterbags accessed by detritivores increased with increasing root litter richness levels at sampling times 1, 3, 4, and 6. At sampling times 2, 5, 7, and 8, it was only larger for the higher root litter richness levels (4- and 8-species mixtures) than at lower richness levels (single species litter and 2-species mixtures) (Table 1). Moreover, detritivores showed a preference for certain plant species, like *Pinus massoniana* and *Syzygium buxifolium* (Fig.S2).

We found that the roots of *Pinus massoniana* and *Syzygium buxifolium* were significantly richer in P and Mg concentrations than most of the other focal plant species (Table S2). In comparison, the roots of *Pinus massoniana* had lower Ca concentrations (Table S2). The partial regression where root richness, detritivore presence, fungal PLFA biomass, bacterial

PLFA biomass, root C/N ratio, root P, Ca, K and Mg, soil moisture and pH were used as explanatory variables, showed that the initial P and Ca concentration had a significant and positive effect on root litter mass loss (Fig. 3). In contrast, other root chemical traits, like root C/N ratio ($P = 0.088$), K ($P = 0.907$) and Mg ($P = 0.133$), had no significant effects.

ROLE OF THE FUNGAL COMMUNITY IN ROOT LITTER MASS LOSS

Soil microbial PLFA biomass was significantly higher at the early stages (i.e. the first four sampling events) than at the later stages of root litter decomposition (i.e. the latter four sampling events). The soil fungal:bacterial ratio increased with decomposing time (Table S1). The partial regression showed that the mass loss of the root litter increased with fungal PLFA biomass (Fig. 3), but did not vary with bacterial PLFA biomass ($P = 0.198$). In addition, both soil bacterial and fungal PLFA biomass were significantly correlated with root fungal community composition (Table 3).

Fungal composition in the root litter at the end of the experiment did not differ between the levels of root litter richness, and was not significantly affected by the presence of detritivores (Table 3). However, there was a significant relationship between the first axis of the 3D-NMDS analysis of the root fungal community and root mass loss ($r = -0.22$, $P = 0.007$). Also, fungal composition depended on the initial root nutrients and soil moisture (Table 3). At the end of the experiment, the fungal communities inhabiting the root litter material of the eight target plant species were dominated by Basidiomycota, and were significantly related to root mass loss (Fig. S4 and Table S3). These results indicate that root-inhabiting fungi have an important role, particularly those from the phylum Basidiomycota, with respect to the later stages of root decomposition. Among the dominant OTUs, there were three species (i.e. *Myxotrichum cancellatum*, Sarcosomataceae sp. and Herpotrichiellaceae sp.) from Ascomycota (Fig. S5). The presence of detritivores caused a significant increase in the

dominant OTUs, including *Myxotrichum cancellatum* (+548%, $P = 0.001$), *Polyporales* sp. (+163%, $P = 0.034$), and *Basidiomycota.sp.2* (+209%, $P = 0.021$) (Fig. S5).

Basidiomycota.sp.1 in the 2-species root litter mixture (+500%, $P = 0.045$), *Mycena.sp.1* in the 8-species root litter mixture (+1102%, $P = 0.013$), *Basidiomycota.sp.2* in the 4-species root litter mixture (+327%, $P = 0.035$), *Russulaceae* sp. in the 2-species root litter mixture (-92%, $P = 0.010$), *Suillus* sp. in the 2-species root litter mixture (+365%, $P = 0.027$), and *Mycena.sp.4* in the 8-species root litter mixture (+284%, $P = 0.006$) were significantly different to those in monospecific roots (Fig. S5). In general, there were no interactive effects of detritivores and root litter richness on dominant litter-inhabiting fungi, except for *Trechisporales* sp. and *Mycena.sp.1* (Fig. S5).

Discussion

The main aim of our study was to test how root litter richness affects the mass loss of root litter. Based on our three-year decomposition experiment, we found that the richness of the root mixtures did not significantly affect the mass loss of fine roots in the absence of detritivores. However, when detritivores accessed litterbags, root mass loss was higher in monospecific root litter compared to mixed root litter, indicating that detritivores might be important for controlling how root litter richness affects root decomposition. The presence of detritivores generally increased root mass loss, particularly during the early stages of decomposition.

The results of leaf litter studies suggest that mass loss may be (though not always) stimulated by plant litter richness, particularly when litters with different functional traits are combined (Gessner *et al.* 2010). In contrast to these findings, we show that the mass loss of roots was not significantly altered by the species richness of root litter. This result indicates that the breakdown of belowground litter might be driven by different, or contrasting,

mechanisms to the breakdown of aboveground litter (Hobbie *et al.* 2010; Berg & McClaugherty 2014; García-Palacios *et al.* 2016). The positive effects of leaf litter mixing are often explained by complementarity effects, whereby resource use is facilitated by resource partitioning or positive interactions (Loreau & Hector 2001). We expected to find similar mixing effects for root litter, particularly because previous work indicated that variation in root chemical characteristics is important for driving root mass loss (Silver & Miya 2001; Freschet, Aerts & Cornelissen 2012; Freschet *et al.* 2013). However, even though the different types of root litter included in our experiment had different chemical compositions, we did not find evidence for increased mass loss due to complementarity effects in the root litter mixtures of our study.

In addition, as microbial communities might mediate complementarity effects, we also expected that diverse litter mixes would result in diverse fungal communities. We observed that more members of the phylum Basidiomycota were involved in the late stages of root litter decomposition than members of the phylum Ascomycota, supporting the general view (Voříšková & Baldrian 2013). However, we did not find any differences in the composition of the root fungal community between the different root richness treatments at the final sampling event. Thus, the role of fungi in driving how litter richness (e.g. in the bags entered by detritivores) affects the rate of decomposition in our experiment appeared to be limited, at least during the late stages of decomposition. Still, local variation in litter mass loss was related to variation in fungal biomass and composition, suggesting that fungal communities are important for root litter breakdown.

We found no evidence that litter richness causes changes in mass loss with decomposition time in our study. One potential reason for this finding is that the equivalent amounts of chemicals in the four root litter richness groups (i.e. initial root C, N, P, Ca, K and Mg; all $P > 0.05$, Turkey's test) might have a minor divergence in the amount of chemicals or

composition with decomposition time. Although root litter richness did not affect the patterns of root litter mass loss over the decomposition period, the positive effect of detritivores on root litter mass loss was stronger during the earlier stages of composition compared to the latter stages of decomposition. One possible explanation is that the litterbags with macro-detritivores were also more preferably accessed by meso- and micro-detritivores, as well as microorganisms (Bradford *et al.* 2002). Moreover, macro-detritivores might prefer root litter material containing more nutritious and fresh organic matter during the early stages of decomposition compared to the later stages of decomposition (García- Palacios *et al.* 2016). Finally, litter fragmentation by macro-detritivores might break the physical barriers of plant tissues, similar to that observed in leaves (Cárdenas & Dangles 2012), exposing easily degradable compounds to microorganisms (Mukhopadhyay, Roy & Joy 2014). This phenomenon might have caused the fast rates of decay during the early stages of decomposition. However, because of the fast decomposition of easily degradable compounds and the relative accumulation of compounds that are more resistant to decay (Coûteaux, Bottner & Berg 1995), decomposers at later stages of decomposition might have had lower resource use efficiency (Wicking *et al.* 2012), decelerating root decomposition.

As predicted in the second hypothesis, we found that the presence of detritivores increased root mass loss. Detritivores commonly speed up root litter mass loss (Liu & Zou 2002; McGlynn & Poirson 2012; Treplin, Pennings & Zimmer 2013) *via* feeding on and fragmenting root litter. Here we show that the presence of detritivores modified how root litter richness affected decomposition. Monospecific root litter lost more mass in the presence of detritivores than root litter mixtures. Our finding provides empirical evidence that detritivores control the root litter richness–decomposition relationship (Hättenschwiler & Gasser 2005), which has rarely been documented for the decomposition of fine root litter (Berg & McClaugherty 2014). The selection of nutrient-rich plant litter containing high N

and P by detritivores might have caused these negative litter richness effects (Swan & Palmer 2006). In our experiment, detritivores mostly consumed root litter types with higher P concentrations, such as *Pinus massoniana* and *Syzygium buxifolium*. Detritivores might prefer these nutrient-rich roots in our study system, due to the widespread N and/or P limitation of biota at multiple trophic levels in subtropical forest ecosystems (Wieder *et al.* 2009; Lin *et al.* 2011). The mass ratio hypothesis states that functional traits and the diversity of dominant plants determine ecosystem functions (Grime 1998; Smith & Knapp 2003; Cárdenas *et al.* 2017). Our experimental design involved a decreasing proportion of root litter material of the plant species *Pinus massoniana* or *Syzygium buxifolium* in mixtures with increasing root richness. The preference of detritivores for these specific plant species might explain the negative effects of root litter richness on root decomposition in the presence of detritivores. Actually, higher root richness enhances the probability of detritivores accessing their preferred plant species, but at lower proportions. This result further indicates the importance of the proportion of specific species, such as *Pinus massoniana* or *Syzygium buxifolium*, in root mixtures, supporting the mass ratio hypothesis.

The detritivore-mediated effects of root litter richness might also follow an indirect pathway, regulating fungal community characteristics. In contrast to what we expected, the lack of an interactive effect of root litter richness and detritivore presence on dominant fungi in our study indicates that the presence of detritivores does not regulate the effects of root richness on root mass loss by affecting litter-inhabiting fungi. However, the non-significant response of the composition of the fungal community does not necessarily mean there was no functional difference (Purahong *et al.* 2014). In fact, the composition of the root litter-inhabiting fungal community and soil fungal PLFA biomass were significantly related, and potentially contributed, to root mass loss in this study. In addition, the fungal community was only measured on the last sampling date, which might not reflect the successional dynamics

and activity of fungi during the decomposition process, because fungal decomposers generally depend on litter quality (Osono 2005) and exhibit rapid species turnover during decomposition processes (Voříšková & Baldrian 2013). Thus, future studies should explore the successional dynamics of root fungi, as well as other microbial groups, on root litter material. Also, in contrast to our third hypothesis, we found that the effect of soil fungal and bacterial PLFA biomass on root mass loss did not depend on the presence of detritivores; thus, the effects of detritivores on root litter mass are not mediated by changes in microbial biomass, at least in the soil studied in our experiment.

Conclusions

In conclusion, our findings demonstrated the important role of detritivores in modifying the root litter richness–root decomposition relationship in subtropical forest ecosystems. We found detritivores altered the richness effect on root mass loss, with this process being dependent on the probability of specific plant species (i.e. *Pinus massoniana* and *Syzygium buxifolium*) occurring in this study, due to being more attractive to detritivores. In addition, this process was dependent on the proportion of these specific component plant species within the root mixture. We did not observe any evidence for the interaction between detritivores and microbial decomposers in affecting the mass loss of root mixtures in the present study. Yet, in general, the relationship between fungal biomass, species composition, and litter mass loss indicates that variation in fungal communities is important for driving local patterns in root litter decomposition. Thus, our results suggest that the indirect effects of detritivores were of minimal importance in modifying microbial decomposer communities in the studied soil. Future studies should investigate the time course of the complex interplay between litter richness, identity, and traits, selective detritivore feeding, and microbial community composition in the decomposition of root litter.

Author contribution

N.Z. conceived the ideas, together with X.M.Z., K.P.M. and Y.L. designed the experiment.

Y.N.L. and X.C. was mainly responsible for establishment of this experiment and collected data. N.Z. and Y.N.L. analysed data. N.Z., G.F.V. and N.E. led the writing of the manuscript.

All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

All data involving in the litterbag experiment are included in the manuscript. The data of initial root chemicals and root mass loss during the experimental duration have been submitted to Dryad (<https://doi:10.5061/dryad.505d9>). The data of OTUs referring to root litter-inhabiting fungi are shown in Table S3. The sequence data obtained from Illumina MiSeq sequencing were submitted to the the GenBank of NCBI (MF789354 - MF794206).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Figure S1 *Schematic illustration of the experimental design.*

Figure S2 *The percentage occurrence of litterbag holes for eight single plant species and seven mixtures.*

Figure S3 *Mass loss of single plant species and their mixtures with and without detritivores.*

Figure S4 *The network of eight focal plant species with root litter-inhabiting fungal phyla.*

Figure S5 *Heat map of the dominant fungal OTUs accounting for at least 1% of the relative abundance.*

Table S1 *Soil and microbial properties during the period of root decomposition.*

Table S2 *The content of initial root nutrients of single and mixed plant species.*

Table S3 *The operational taxonomic unit (OTU) matrix of fungi inhabiting root samples.*

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Tables

Table 1 Relative proportion of litterbags accessed by detritivores of the total number of litterbags of each root litter mixture treatment per sampling time. Single, Two, Four and Eight refer to single plant species, two-, four- and eight-species root mixture.

Decomposing time (days)	Month	Occurrence of litter bags with holes (%)				
		Single	Two	Four	Eight	Total
97	Jan	13.0	23.9	34.8	50.0	21.4
170	Apr	14.6	31.3	20.8	50.0	22.2
278	Aug	17.9	23.4	29.2	33.3	21.9
357	Oct	31.3	41.7	50.0	75.0	39.4
482	Feb	17.7	39.6	33.3	41.7	27.2
737	Oct	22.1	30.2	36.4	40.0	27.3
826	Jan	14.9	29.2	29.2	27.3	21.5
921	Apr	19.1	12.5	20.8	33.3	18.5

Table 1 Effects of decomposing time, root richness, and detritivore presence on root mass loss based on a linear mixed effects model with decomposing time, root richness, and detritivore presence as fixed factors and plot nested in block as a random factor. The bold values indicate significant effects on root mass loss.

Source	df	Sum of Sq	Wald statistic	<i>Pr</i> (Chisq)
Time (T)	7	684	921	< 0.001
Richness effect (RE)	3	3.10	4.20	0.244
Detritivore effect (DE)	1	611	823	< 0.001
T×RE	21	14.2	19.0	0.582
RE×DE	3	31.3	42.1	< 0.001
T×DE	7	64.9	87.3	< 0.001
T×RE×DE	21	15.3	20.5	0.487

Table 3 Three-dimensional non-metric multidimensional scaling (3D-NMDS) results of fungal communities living on decomposing root litters. C/N = the ratio of root carbon to nitrogen, P = root phosphorus, Ca = root calcium, K = root potassium, Mg = root magnesium, SM = soil moisture at 0-10 cm depth, pH = soil pH, Fungi = fungal PLFA biomass, Bacteria = bacterial PLFA biomass. The bold values indicate significant association of root-inhabiting fungal community composition with root initial chemicals and soil microbial PLFA biomass.

	NMDS1	NMDS2	NMDS3	r ²	Pr(>r)
Richness effect	-0.057	-0.468	0.882	0.014	0.539
Detritivore effect	-0.493	-0.282	0.823	0.021	0.378
C/N	0.342	-0.938	-0.049	0.060	0.035
P	-0.431	0.603	0.671	0.154	0.001
Ca	0.067	0.123	-0.990	0.068	0.014
K	-0.260	0.585	0.768	0.070	0.013
Mg	-0.400	0.485	0.777	0.029	0.200
SM	-0.874	0.322	-0.365	0.167	0.001
pH	-0.736	0.673	0.077	0.015	0.523
Fungi	0.008	0.917	-0.399	0.072	0.008
Bacteria	-0.393	0.852	-0.346	0.098	0.002

Figures

Figure 1 Effects of fine root litter species richness (diversity levels) on mass loss with or without detritivores. Single, Two, Four and Eight refer to single plant species, two-, four- and eight-species root mixture. Different letters in upper case represent significant differences of mass loss among richness levels in the presence of detritivores, and different lower case letters refer to significant differences in the absence of detritivores ($P < 0.05$, Tukey's test).

Error bars represent standard errors.

Figure 2 Temporal dynamics of root mass loss with or without detritivores. Different letters in upper case represent significant differences of mass loss among sampling times in the presence of detritivores, and different lower case letters refer to significant differences in the absence of detritivores ($P < 0.05$, Tukey's test). Error bars represent standard errors.

Figure 3 Relationships from partial regressions between the residuals of root litter mass loss and (a) root P ($P < 0.001$), (b) root Ca ($P = 0.037$), (c) fungal PLFA biomass ($P < 0.001$), and (d) soil moisture ($P < 0.001$) (see main text for details).