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Article

Fine Roots of *Parashorea chinensis* Decompose Slower than Twigs

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Abstract: Plants produce above- and below-ground biomass. However, our understanding of both production and decomposition of below-ground biomass is poor, largely because of the difficulties of accessing study materials. Below-ground organic matter decomposition studies are scanty and especially rare in the tropics. Here, we used a litter bag experiment to quantify the mass loss and nutrients dynamics of decomposing twigs and fine roots from an arbuscular mycorrhizal fungal associated tree, *Parashorea chinensis*, in a tropical rain forest in Southwest China. Overall, twig litter decomposed 1.9 times faster than fine roots (decay rate (k) twig=0.255, root=0.134). The difference in decomposition rates can be explained by a difference in phosphorus (P) concentration, availability and use by decomposers or C quality. Both materials showed an increase in N concentration, with final measurements still higher than initial levels. This suggests N may not be available due to microbial immobilization. Both carbon and nitrogen dynamics were significantly predicted by mass loss and showed a negative and positive relationship, respectively. Our study results imply that fine roots carbon and nitrogen contribute more to soils organic matter and enlarge the resident time. Therefore, better understanding of carbon cycle requires better understanding of mechanisms governing below ground biomass decomposition.

Keywords: Fine root; tropical rainforest; nutrients dynamics; litter bags; decay rate; nitrogen mineralization, Calcium, Magnesium.

1. Introduction

Litter production and decomposition represent the principle pathways of carbon and nutrient cycling in terrestrial ecosystems. However, these pathways are

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much better understood for the above-ground organs, as compared to below-ground ones [1]. This is mostly due to difficulties related to accessing and sampling below-ground litter materials [2,3]. From studies on above-ground litter materials, we know that decomposition is controlled by both abiotic and biotic factors [4,5].

While the main drivers of decomposition vary with respect to the litter types across biomes and among organs, litter quality is consistently one of the most important factors controlling litter decomposition [4]. A cross continental meta-analysis found that C:N ratio, Ca, and lignin:N ratio were the most important determinant of root litter decomposition [6,7]. For example, Sun and colleagues recently showed that there was a contrast in the main quality traits that govern the decomposition of leaf litter and fine roots. Whereas lignin:N, C:N ratio, Mg, N, and Mn content were important determinants of leaf litter decomposition, none of these traits explained the decomposition of fine roots [8]. Meanwhile, a recent study found condensed tannins were a critical determinant of fine root decomposition [9]. On the other hand, for twig decomposition, recent research showed that C and P are important drivers of twig decomposition, with C exhibiting a negative relationship and P showed a positive one [10]. When compared to root decomposition, of the few studies that exist, beech twigs were found to decompose faster than roots in a temperate forest and this was attributed to ligninolytic enzyme inhibition by certain N compounds to favor the formation of N rich humus [3].

Studies often report the initial chemical composition of decomposing litter, including those on roots and twigs. However, studies rarely monitor the dynamics of nutrients during the course of decomposition [11]. Initial litter chemistry also depends on the size for the component plant organs, and litter size is negatively associated with decomposition rates [12]. This is partially due to the positive relationship between size and concentrations of recalcitrant constituents [11,13]. Relatively few studies have investigated the decomposition of different plant organs while controlling for litter size.

Different organs are specific with regard to the function they perform, which in turn controls their chemistry [14]. Twigs and fine roots are the most distal parts of a tree. Twigs serve to mechanically support the leaves and as storage organ for nutrients and carbon. These are often reabsorbed by plants during leaf senescence and also allocated to the developing fruits during reproduction [15]. Whereas, fine roots are responsible for water and nutrient absorption at the plant soil interface [16]. After the senescence of organs, they show specific way in decomposition [10]. Some reports indicate that twigs decompose slowly as compared to coarse woody debris and the presence of bark in twigs generally inhibits decomposition. Despite the large number of studies on the decomposition of terrestrial woody debris, relatively few have considered twig decomposition [3,10,14,17]. Moreover, there are a large number of studies on single organs or tissues, but far fewer on comparisons among organs (leaf vs. root, leaf vs. stem, stem vs. root etc.). For

example, even though fine roots (first order roots) exhibit short life spans, they have longer carbon residence times than larger diameter roots, at least in the temperate and sub-tropical biomes [18] with a handful studies in the tropics [1,19,20]. However, the mechanisms underlying the slow decomposition of fine roots are poorly understood [21]. To date, four potential hypotheses have been proposed that attempt to explain the mechanism behind slow fine root decomposition.

The first hypothesis, or mycorrhizal hypothesis, stipulates that fine roots are protected by chitin formed by ectomycorrhizal (EM) fungi which impedes decomposition [22]. The second hypothesis, called the "C quality" hypothesis, states that regardless of whether trees are colonised by EM or arbuscular mycorrhizal (AM) fungi, tree roots harbor a higher proportion of insoluble or unhydrolizable components (e.g., suberin, tannins and lignin) leading to slow decomposition. The third hypothesis concerns N concentration, the so-called "N inhibition" hypothesis, and posits that the high N content of fine roots combines with acid insoluble C to induce the formation of recalcitrant constituents [18]. Finally, the fourth hypothesis, attempts to reconcile the previous three, by suggesting that the fungal association determines the mechanism, and is called the "myco-quality" hypothesis [23].

The above hypotheses were derived from the existing work on fine root decomposition which is largely restricted to temperate and subtropical species [8,10]. However, there is need to understand whether these hypotheses remain valid across biomes and therefore can be generalized [23].

In this study, we used a standard litter bag study to investigate the decomposition of twigs and fine roots (<5 mm in diameter) in a tropical tree. We standardized the size (< 5 mm in diameter) of litter materials and aimed to elucidate (i) the decomposition patterns of fine roots and twigs of *Parashorea chinensis*, a tropical AM tree, (ii) the mechanistic explanation behind the contrasting patterns of twig and root decomposition, and (iii) the nutrients dynamics of these two litter materials.

2. Materials and Methods

2.1. Study site

This study was conducted at the *Parashorea chinensis* forest national park in Mengla county, Xishuangbanna, Yunnan Province, China (21°37′39″ N, 101°35′24″ E; 650 m above sea level) (Figure 1). The slope and aspect of the study site are 20-25° and SW15°, respectively. The climatic conditions of this forest are typical monsoonal conditions. According to the long term data of Mengla meteorological station situated approximately 20 km from the study site, the 40-year mean annual temperature was 21.3 ℃ (ranging from a minimum of 15.8 ℃ in January to a maximum of 25.6 ℃ in June). Annual mean precipitation is 1511 mm and on

average 85% of this precipitation occurs during the rainy season from May to October. Latosol soils are dominant at this research site and have an average pH of around 5.5. The soil organic matter in the top 10 cm layer comprises ~2.91 % whereas for the deep layer (10 -25 cm) it is around 2.01 %. The dominant vegetation type is lowland tropical rain forest-*Parashorea chinensis* forest, which is a type of mono-dominant rain forest [24,25]. Such forest is rich in plant species and shows a complex forest canopy structure. Briefly, the forest canopy can be divided into four sublayers. The emergent layer is dominated by *Parashorea chinensis* with umbrella crowns emerging sparsely from the main canopy up to 60 m in height. The second layer ranges from 30 to 40 m in height and is rich in tree species, including especially *Pometia tomentosa*, *Garcinia cowa*, *Ficus langkokensis*, *Knema furfuracea*, *Barringtonia macrostachya*, *Pseudouvaria indochinensis*, *Gironniera subaequalis* and *Cinnamomum bejolghota*. The mid-canopy layer ranges from 20 to 30 m and typical species include *Baccaurea ramiflora*, *Dichapetalum gelonioides*, *Chisocheton siamensis*, and *Diospyros atrotricha*. The understorey layer ranges from 5 to 15 m and is typically hosts *Pittosporopsis kerrii*, *Phoeba lanceolata*, *Cleidion bracteosum*, *Syzygium latilimbum*, and *Drypetes indica*. Details of the plant species composition and community characteristics of the forest was described in detail by Zhu [24].

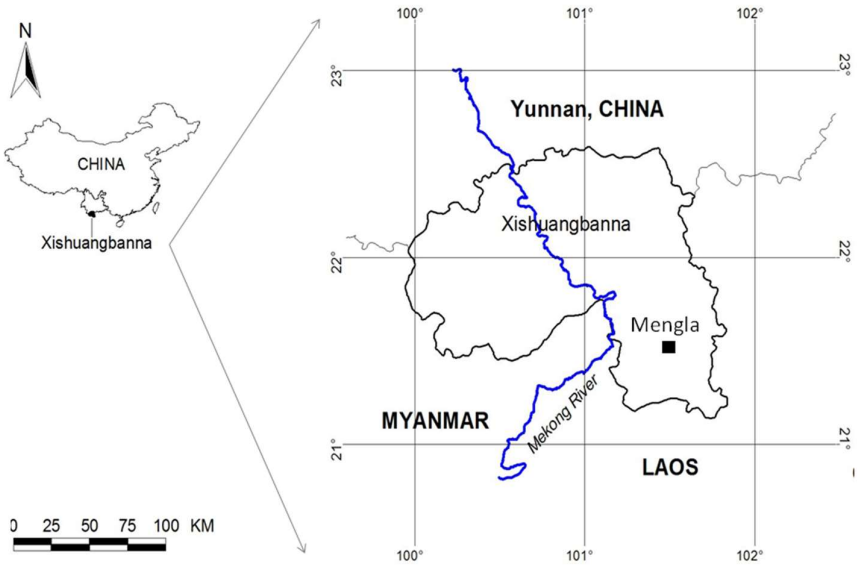

Figure 1. Study site location

2.2. Litter materials and litter bag preparation

In 2003, we uprooted five recently felled (within a week informed by park regular guards) individuals of *Parashorea chinensis* , and partitioned the biomass of these trees into the above- and below-ground fractions and by organ. The collected litter materials were used to study their decomposition. To monitor litter decomposition

of twigs (<5 mm in diameter) and fine roots (<5 mm in diameter), we used nylon litter bags of size 20 cm by 30 cm for twigs (length= 10 cm) with 1 mm mesh size (50 g fresh litter per bag) and 20 cm by 15 cm with a mesh size 0.5 mm for root (10 g fresh litter per bag). Such mesh sizes while allowing meso- and micro-fauna to enter the litter bags, excludes macro-invertebrates including, for example termites [26]. The different sizes and mesh size of the bags could potentially affect the rate of decomposition, which we discuss in the Discussion section [27]. We established a small common garden litter bed of size of 100 m x 50 m. Litter bags for twigs were incubated on the forest floor while those for root decomposition were buried at 20 cm in the soil. Litter bags were placed 5 m intervals. We employed five replicates per harvest time and have 12 harvest times (90, 181, 273, 360, 457, 545, 639, 731, 821, 912, 1004, 1093 days incubation). Therefore, in total, 120 litter bags [(5 replicates x 2 sample type (fine root and twig) x 12 harvesting times)] were used. Additional litter material was used to calculate moisture content and dry weight of the initial materials. Twig litter bags were filled with on average 33.7 ± 0.002 g, while fine root litter bags were filled with 7.62 ± 0.002 g. At harvesting, there was no evidence that termites entered any of the litter bags. At each collection, five replicates of each litter material were retrieved from the forest floor, and transported in ziplock plastic bags to the laboratory. Upon arrival in the laboratory, every litter bag was gently cleaned to remove any soil and herbs or grass roots, and the remaining material in the litter bag was gently washed with tap water before transferring it to a paper envelope. Litter material was oven dried at 105 ^{o}C to constant mass. Measurements of mass were made at 0.01 g precision with an electronic balance.

2.3. Chemical analysis

At the initial installation of the litter bags, an extra materials of each litter type were used after filling litter bags in the laboratory to determine water content. Afterwards, these were ground into powder before chemical analysis. Likewise, at each harvest the litter material remaining in the litter bags was dried and analyzed for chemical content. For these analyses, materials from replicates of the same litter type were pooled together to form a composite sample to reduce the costs of the chemical analysis. Analyzing the chemical content at each harvest time enables monitoring of the dynamics of nutrients during the decomposition process. Total carbon (C), nitrogen (N), phosphorus (P), potassium (K), Calcium (Ca), magnesium (Mg) contents were determined using standard methods. Briefly, C and N were quantified with a Vario Max CN element analyzer (Elementa Analsensysteme, Germany). For P and K, samples were mixed with HCl and digested with HNO_3-$HClO_4$ and measurements were made using an inductively coupled plasma (ICP) atomic emission spectrometer (iCAP 6300, IRIS Advantage, E R, Thermo Fisher Scientific, USA). For Ca and Mg, samples were prior digested with both H_2O_2 and concentrated HNO_3 and afterwards their Ca and Mg concentrations were

measured by a Thermal Jarell ICP (Scientific Instrument Services, Inc., Ringoes, NJ, USA).

2.4. Data analysis

We used a single negative exponential model with the decay constant rate *k* [28]. Though we acknowledge the issues related to the use of such model, we believe the small size of the litter material involved in our current experiment fully supports its use [29]. For computing the decay rate constant for both fine roots and twigs, we used the following equation [30]:

$$M_t = M_o * e^{-\left(\frac{k*t}{365.25}\right)} \quad (1)$$

Where M_t represents dry mass at harvest time t, t is the number of days incubation, $M_o$is the dry mass at time zero, 365.25 converts days to years, and k is the decay rate constant (per year). We compute *k* by employing non linear least squared (nls) regression using the function *nls* implemented in the "nlme" package in R [17,31]. We calculated percentage mass loss for each bag at each harvest. We used a generalized least squares (function *gls* in the "nlme" package) to model the percent mass loss (logit transformed) from twigs with that from fine roots. We employed the argument variance identity (varIdent) function to allow variances to change with regards to sample type (twig and fine root). We performed a principal component analysis (PCA) with the function *prcomp* to determine the chemical elements that explain most of the variation in the concentrations of chemical elements across samples. We also used linear modeling with the function *lm* to assess the relationship between carbon and nitrogen concentrations and percentage mass loss. In order to monitor the dynamics of C and N through decomposition, we computed the fraction of initial C and N remaining [3,21]. All the statistical analyses were done using R version 3.4.3 (R-Foundation for statistical computing [32].

3. Results

3.1. Decomposition dynamics and decay rate

By the end of the experiment, the percentage mass loss for twigs and fine roots was on average 97% ± 0.98% (94.2 - 99.1 %) for twigs and 82.2% ± 4.65% (80.7 - 89.6 %) for fine roots. Overall, twig litter decomposed 1.9 times faster than fine root litter (decay rate twig=0.26 *vs.* root=0.13) (Table 1, Figure 2). However, the variation in fine root decomposition was larger than that for twig decomposition.

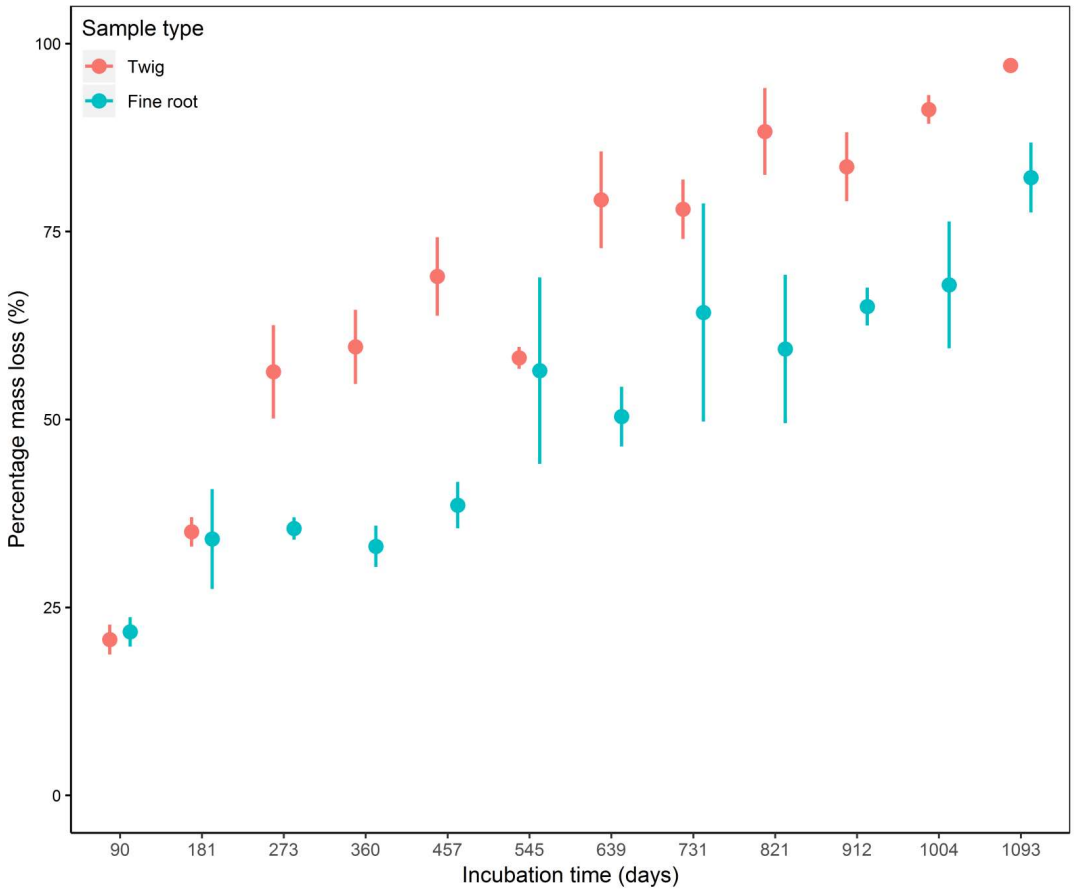

Figure 2. Percentage mass loss for twig and fine root litter of *Parashorea chinesnsis* litter monitored through a litter bag experiment for 1093 days in Mengla county, Xishuangbanna, China. Red = twigs; Blue = fine root. Dots represent the mean for samples collected at each harvest and errors bars represent the standard errors (n=5).

3.2. Initial chemistry and nutrient dynamics during decomposition

The initial concentrations of N, P, K, Ca, and Mg were higher in twigs than in fine roots (Table S1), and C concentration and C:N ratio were lower in twigs than in fine roots. Looking at the chemical dynamics through the decomposition process, the magnitude of C, P and K loss was higher in twigs than in fine roots, and the magnitude of the relative increase in N and Mg was higher in twigs than in fine roots. In both materials the C:N ratio declined up to 1004 days incubation and thereafter started to increase (Table S1, Figure S1). For the principal component analysis (PCA), we found that the first two axes combined explained almost all (91.49 % with PCA1 explaining 61.56 % *vs.* PCA2 29.93 %) the variation in the litter chemistry, as measured from the initial litter through to the end of the decomposition process (Table S1, Figure 3). C, Ca, N, Mg, and K were the most important elements in explaining variation chemistry among the litter materials through time.

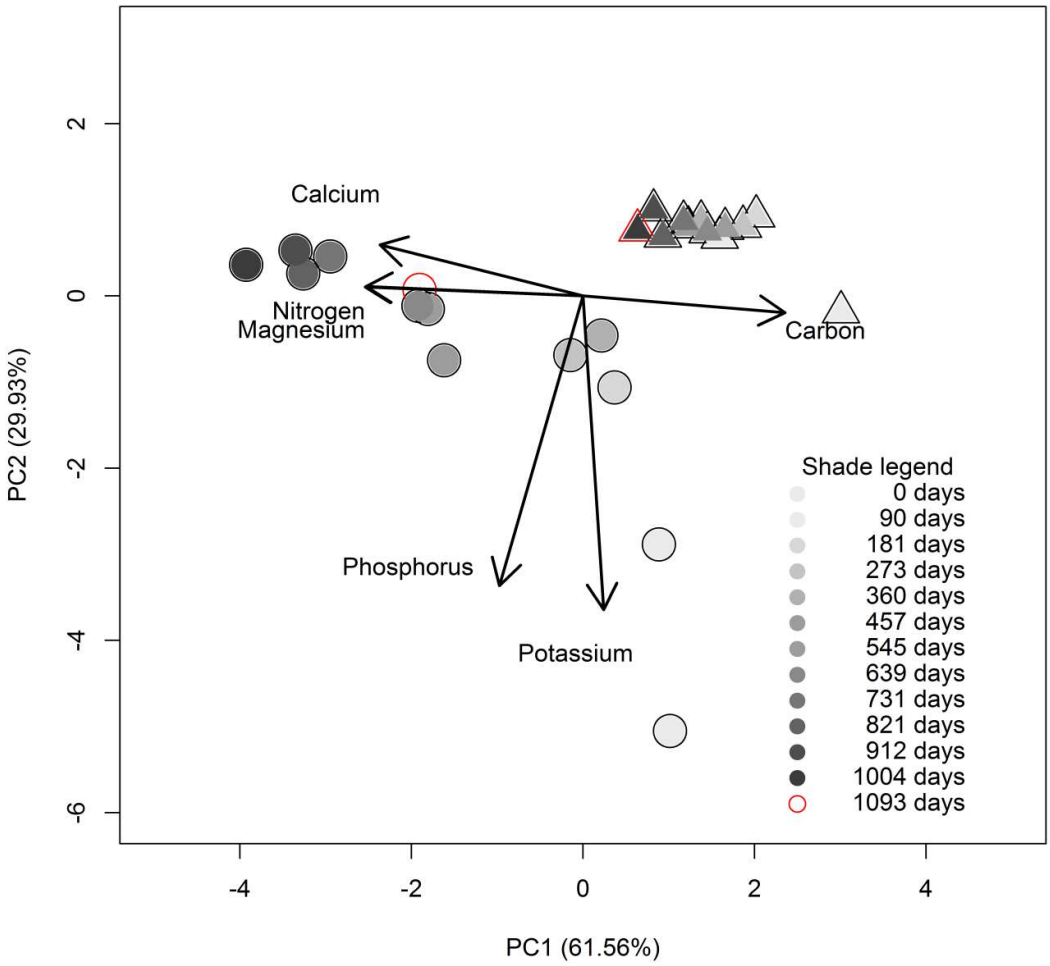

Figure 3. Principal components analysis (PCA) of the chemical contents of twig (circle symbol) and fine root (triangle symbol) litter from *Parashorea chinensis* through the decomposition process. The degree of shading indicates how long the materials have been incubated. The darkest grey represents the starting point (0 days) and empty symbol the last collection (after 1093 days incubation). Materials were incubated in a common litter-bed on the rain forest floor at Mengla county, Xishuangbanna, China. Note that the contribution of Magnesium and Nitrogen in explaining the variation of nutrients concentration during decomposition is the same so their respective arrows are overlapping. Also due to overlapping, we use red color to show the last collection.

The C concentration dynamics of decomposing twigs and fine roots were similar during decomposition. However, the decline in C concentration was more pronounced in twigs than in fine roots. By the end of the experiment, twigs had lost 25 % compared to 21.5% for roots of the initial fraction (Figure S1). With regard to N, there was a marked increase in N concentration through the decomposition process. Three phases in N dynamics for roots: increase phase (0 to 360 days), stabilization phase (360 to 731 days) and second increase phase (731 to 1093 days). For twigs, two phases are distinguishable: increase phase (0 to 912 days) and decrease phase (912 to 1093 days). From 360 days until 912 days incubation, the rate was more pronounced in twigs than in fine roots. However, soon after this

point (912 days), the N concentration declined sharply in twigs and was stable for fine roots. At the end of the monitoring period, the N concentration in both materials reached more or less the same level but was still 193 % and 194 % of initial levels for twigs and fine roots, respectively (Figure S1).

3.3. Relationship between decomposition and C and N concentrations dynamics

An increase in percentage mass loss is inversely linked to C concentration. With a given mass loss inducing a more substantial decrease in C in the twigs than in roots. Conversely, N concentration is positively associated to percentage mass loss. Again as decomposition proceeds, the increase in N concentration is higher in twigs than in roots (Figure 4).

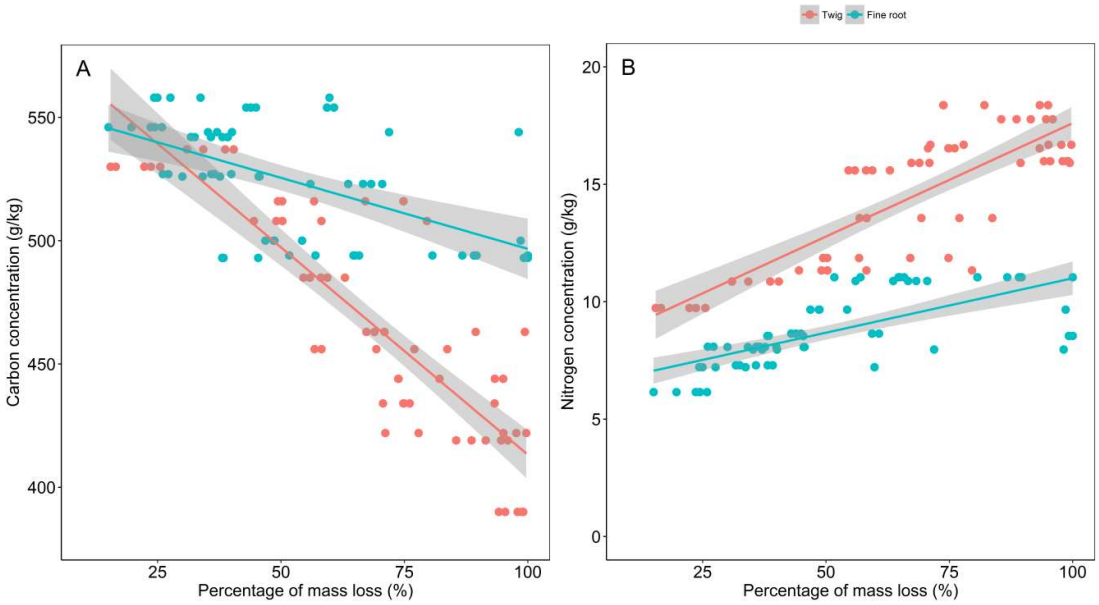

Figure 4. Relationship between percentage mass loss and (A) carbon concentration and (B) nitrogen concentration for twigs (red dots) and fine roots (blue dots) of *Parashorea chinensis* over 1093 days in Mengla county, Xishuangbanna, China. Dots represent mass loss from each litter bag but chemical concentrations were analysed from pool samples (n=5) of the litter bags harvested at each point in time.

Table 1. Comparison of percentage of mass loss (logit transformed) from twigs and fine roots of *Parashorea chinensis* during decomposition monitored in a tropical rain forest in Mengla county, Xishuangbanna,China over a period of 1093 days. Percentage mass loss (logit transformed) was modeled as function of incubation time (number of days), sample type (twigs and fine root) and two-way interaction. The intercept includes the baseline level of sample type (twig).

Parameters	Estimate	Standard error	t-value	p-value
(Intercept)	-0.769	0.142	-5.406	0.000
Incubation time	0.002	0.000	11.369	0.000
Fine root	-0.229	0.201	-1.140	0.256
Incubation time:Fine root	-0.001	0.000	-2.067	0.041

4. Discussion

In the present study we set up a standard litter bag experiment to examine the patterns of twig and fine root decomposition in an AM tree, *Parashorea chinensis*, in a tropical rain forest in SW China. We also monitored nutrient concentration dynamics in the decomposing litter over a three year period. We found that twigs decomposed faster than fine roots and that C and N concentration had a negative and positive relationship, respectively, with percentage mass loss regardless to the litter substrate.

4.1. Decomposition dynamics and decay rate

Twigs decomposed faster than fine roots in our study and the percentage mass loss at the end of the experiment were 97 % (k= 0.26) and 82 % (k= 0.13), respectively. This finding is consistent with some other reports from temperate biomes [3,10,14] and from tropical biomes [1,19]. However, this does not corroborate the finding from Garrett et al 2012, where roots decomposed faster than stems and branches (these were large in size compared to ours). [1](Lalnunzira & Tripathi 2018)[19](Bloomfield et al. 1993)In terms of decay rates for roots, our values are much less than the ones reported for *Hopea* roots in Thailand (dry evergreen forest), which had a k between 0.55 and 1.27 [20], and in India [0.66-1.16] [1]. However, these studies used larger root diameters. Similarly for twigs, our measured decay rate was less than previously reported for branches in India [0.55-0.66] [1]. Among the previous studies, to our knowledge only one controlled for the size (diameter) of litter material, and they found that across the three different sizes they used, roots decomposed slower than twigs [3]. The difference in the decomposition rate of twigs and roots might arise for several reasons. First, it could be related to the contrasting use of P by decomposers. Indeed, the initial concentration of P in twigs is ~6 fold higher than that of roots. By the end of the experiment, the magnitude of the difference was only 2 fold, with the P concentration in roots relatively unchanged through the decomposition process. It has been suggested that the availability of P potentially limits decomposition, especially root decomposition [19].

Second, although there was initially more carbon in fine root litter, this carbon seems to be poorer in quality or unavailable, perhaps because this carbon includes some from the root's AM fungi which is usually poor in quality. This supports the "C quality" hypothesis in explaining why fine roots decompose much slower than twigs [18]. Also it is possible that the structure of the decomposing material affects the rate of decomposition, but such mechanisms are not well understood at the moment. Third, slower decomposition of roots compared to twigs might be due to inhibition of saprophytes by mycorrhizal fungi [23]. Moreover, the same author argued that a protein named glomalin, a hydrophobic constituent of AM fungi, might cause slower decomposition of fine roots. Fourth, the higher concentration of N in twigs compared to fine roots is also a potential explanation. Indeed,

previous studies suggest that the higher the initial N concentration, the faster the decomposition [26].

As mentioned in the methods section, the difference in mesh size we used could have direct and also indirect effects on decomposition rates [27]. Whereas the twigs which were incubated in litter bags with a mesh size of 1 mm our root litter was incubated in bags with 0.5 mm mesh. However, fraction of meso-fauna that could enter a 1 mm mesh bag, but not a 0.5 mm bag, is quite small and does not include any functional groups that are considered important in the decomposition of woody materials. Termites are the most important decomposers of woody material, among macro- and mesofaunal components, in sub-and tropical forests and there was no evidence that termites entered the bags. Hence, it seems unlikely that the differences found in decomposition rates between twigs and fine roots were an effect of differences in fauna consumption generated by the difference in litter bag mesh size. Although this is not the first time different mesh sizes have been used to compare the decomposition of different organs (see [10,14]), in the future care should be taken to standardise litter bags when the intention is to compare decomposition rates among organs.

4.2. Initial chemistry and nutrients dynamics during decomposition

Higher initial N in roots compared to other plant organs is commonly reported [1,33]. However, we found the opposite in our study with the initial nitrogen concentration of fine roots being lower than that of twigs in *Parashorea chinensis*. Lower N in fine roots may indicate a strong limiting factor for decomposers [21]. However, there was an increase in N concentrations in both materials up to 1004 days incubation. N concentration after 36 months incubation for beech and ash was in the range of 150-225% of the initial levels for twigs and 90-125% for fine roots [3]. In our experiment it was around 180% of initial levels in both materials. These dynamics in N concentration through the decomposition process reflect nitrogen translocation from surrounding environment into the decomposing materials. Other authors have found that N levels revert to the initial level after 3 years of monitoring [34].

Conversely, fine roots showed higher concentrations of C than that of twigs. Lalnunzira & Tripathi (2018) found a consistently higher C concentration in branches (>10 mm in diameter) than in fine roots in Indian tropical, successional forests, which varied in age from 5 yrs to mature forest [1]. The difference between their findings and ours may reflect the difference in the size of litter materials. Our twigs were 5 mm diameter, whereas the branches they studied were over 10 mm diameter. In our study, the value of the initial C:N ratio was 98 for roots and 63 for twigs. Theoretically, a C:N ratio higher than 75 results in slow decompose due to a higher content of recalcitrant constituents, such as lignin and tannin [6]. This may partly explain the difference in decomposition rates observed between fine roots and twigs. Our value for root was higher than that reported for forests in India,

where the value ranged from 38 to 68, but twigs were of a similar value to that for branches (10 mm diameter) in the Indian study (62-64) [1]. In both materials, the C:N ratio continuously declined through the decomposition process up to 1004 days incubation, as has been found in other studies [26], but thereafter the C:N ratio increased.

Finally, it was interesting to see the potential importance of Ca and Mg in explaining the variation in nutrient dynamics in both fine root and twigs. This reflects the important role of these elements in decomposition [35,36].

4.3. Relationship between decomposition and C and N dynamics

We found that C concentration was negatively correlated with percentage mass loss, whereas N concentration was positively correlated with percentage mass loss. There was also a significant effect of material type. Twigs showed the most pronounced decline and increase, respectively. A study of *Pinus radiata* decomposition in New Zealand found that C concentration did not vary with mass loss, but N concentration increased with percentage mass loss [37]. While studying the dynamics of carbon in roots and branches (larger size than ours) these authors did not find any significant difference among organs, but there was an overall decline in C concentration during decomposition. The dampened C dynamics in fine root in our study may reflect differences in way C is immobilized compared to coarse roots. Another study from temperate biome also observed a rapid accumulation of N in twigs compared to fine roots [3]. However, some studies have not found a monotonic relationship between N dynamics and mass loss. Instead, they found an increase in N concentration up to a peak after 6 months, and thereafter a decline [1]. One study even found a consistent decline in N concentration with mass loss [20].

5. Conclusions

We monitored the decomposition of twigs and fine roots over three years for a tropical AM tree. We found that twigs decomposed faster than fine roots, although initial C concentration was higher in fine roots. Both materials showed an increase in N concentration which did not revert to initial levels even after three years incubation. Whereas the C concentration of litter materials decreased throughout the monitoring period, with a more pronounced decrease in twigs. The difference in P use, as well as potential poor quality of C in roots may explain the differences found in the decomposition rates of fine roots and twigs. Percentage mass loss significantly predicted the dynamics of both C and N concentration throughout the course of decomposition. Our finding supports the “C quality” hypothesis and also points to the importance of Ca and Mg in driving the decomposition of organic materials. Further study is needed to confirm these findings among other tree species in tropical biomes and will help to generalize our current understanding of root decomposition, as well that of other neglected organs. Greater understanding

of below and above of biomass turnover is needed to improve our current models of C and nutrient cycling.

Supplementary Materials: Figure S1: Temporal dynamics in the concentration of carbon (C) and nitrogen (N) in litter materials of *Parashorea chinensis* monitored for 1093 days in Mengla county, Xishuangbanna, China, Table S1: Initial chemistry and their dynamics in twig and fine root from *Parashorea Chinensis* litter monitored during the course of decomposition (for 1093 days) in Mengla county, Xishuangbanna, China.

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