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# Root-order-associated variations in fine-root decomposition and their effects on soil in a subtropical evergreen forest

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## Abstract

**Background:** Despite the importance of root decomposition in predicting ecosystem responses to future climate change, the effects of branch order on root decomposition and the feedback to soil still remains poorly understood. Here we separated root samples taken from two tree species (*Castanopsis fargesii* and *Schima superba* in subtropical forests along the coastal area in eastern China) into four-order classes (1st–2nd order, 3rd order, 4th order, and 5th order) and conducted a 540-day litterbag incubation experiment in laboratory to examine root mass loss, nutrient release, and the influence on soil during decomposition.

**Results:** *C. fargesii* roots of 1st–2nd and 3rd order decayed more slowly than those of 4th and 5th order, but this pattern was not significant for *S. superba*. Of all the measured root traits, the decomposition rates correlated best with root C/N ratio, diameter and specific root length (SRL) based on the structural equation modeling. Both tree species and root order exhibited significantly effects on root initial traits. Overall, *C. fargesii* roots decay faster than *S. superba*, and this appears to be associated with root initial C quality and N concentration. In addition, root order positively affected root decomposition rates mainly through root diameter and SRL. However, no significant difference was found in C and N content between soils below the litterbag with different-order roots.

**Conclusions:** Our findings suggest the effects of branch order on root decomposition are dependent on tree species. Moreover, root morphological properties might also be the controlling factor in root decay besides root chemistry fractions. Overall, the integrative effects should be considered to improve our understanding of the fate of fine-root litter and their contribution to soil C and N pool.

**Keywords:** Root branch, Mass loss, Initial quality, Subtropical evergreen tree, Soil carbon

## Introduction

Plant litter decomposition is a critical process in ecosystem soil organic matter formation and nutrient dynamics (Berg and McClaugherty 2014). According to a global estimation, fine-root litters accounted for 41% of annual litter inputs in forests (Freschet et al. 2012). Since fine roots are responsible for water and nutrient acquisition,

they can rapidly adjust their biomass to the altered resource availability in space and time with a relative short lifespan (Pregitzer 2002; Trumbore and Gaudinski 2003). Such character of life form directly influences carbon (C) and nitrogen (N) cycling in the plant–soil system (Bardgett et al. 2014; Carmona et al. 2021). These fine roots represent about 30% of total global annual net primary productivity if they turn over only once per year and the percentage is even up to 40–50% in forest ecosystems (Vogt et al. 1996; Jackson et al. 1997). Given recently increasing evidences of slow decomposition of fine roots, litter from fine roots may represent a larger contribution to stable soil organic matter (SOM) accumulation (Lin

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and Zeng 2017). Moreover, the amount of N returned to the soil from fine-root death and decomposition equals to or exceeds that from leaf litter fall (Vogt et al. 1986; Gordon and Jackson 2000). However, far fewer studies of litter decomposition focused on root than on leaf litter (Zhang et al. 2008; Silver and Miya 2001; Sun et al. 2018; Chen et al. 2021).

Numerous studies suggest that leaf litter decomposition is controlled by climate, leaf C/N ratio, and lignin/N ratio (Bradford et al. 2016), and this information has already been incorporated in global C models (Adair et al. 2008). However, it seems still far from summarizing general patterns in root litter decay at large spatial scales based on much fewer studies in comparison with above-ground litter (Birouste et al. 2012; Ma et al. 2016; Jo et al. 2016). The heterogeneous results in fine-root decomposition were partly due to the classification method of fine roots in different studies (Lin and Zeng 2017). Diameter was traditionally used to classify fine roots. With this criterion, some researchers reported that very fine roots (<0.5 mm) decomposed more slowly than 0.5–2.0 mm roots (Sun et al. 2012, 2013), while others found that thinner roots decomposed faster (Makita et al. 2015). These contrasting conclusions might be attributed to the large variation in root traits within the same diameter class. Indeed, tree fine roots (<2 mm diameter) comprise multiple branch orders differing markedly in morphology, nutrient components, and functions (Pregitzer et al. 2002; Wells et al. 2002; Xia et al. 2010). The pattern that lower order roots decomposed more slowly than higher order roots seems to be common in studied tree species (Fan et al. 2010; Goebel et al. 2011; Xiong et al. 2013; Sun et al. 2016). However, to our knowledge, almost all these studies examining root decomposition by branch orders focused on temperate forest tree species. In the only two studies on subtropical tree species, one showed that two species out of four differ significantly between lower order and higher order roots (Xiong et al. 2013), the other demonstrated large variation in litter decomposition rates across root orders among four litterbag methods (Chen et al. 2021). Yet, it's notable that lack of studies on root decomposition based on root-order classification for tree species from different forest ecosystems constrains the establishment of the principle that lower order roots decay more slowly than higher order roots globally.

The potential mechanisms to explain the slower decomposition of lower order roots have been identified to three main hypotheses (Fan et al. 2010; Beidler and Pritchard 2017). For C quality hypothesis, lower order roots contain higher acid-insoluble residue and lower total non-structural carbohydrate (TNC) resulting in their slower decomposition rates (Fan et al. 2010; Guo et al. 2008a). For N inhibition hypothesis, the higher N

content may inhibit root decomposition of lower order roots with high acid-insolubles (Berg and McClaugherty 2014). This is because high N suppresses the activity of lignin-degrading enzyme (ligninase) or promotes humus formation (Berg and McClaugherty 2014; Magili and Aber 1998; Sinsabaugh et al. 2002). For mycorrhizal hypothesis, lower order EM roots, which are preferentially colonized by mycorrhizal fungi to higher order branches (Guo et al. 2008b), would have thick fungal sheath rich in chitin, a recalcitrant compound with a high N concentration, decayed slower (Langley and Hungate 2003). Thus, root decomposition rates were found to be positively correlated to initial TNC and negatively related to N concentrations (Fan et al. 2010; Goebel et al. 2011). However, others found there was no relationships between root decomposition rate to litter initial N content or C/N ratio (Xiong et al. 2013). Moreover, arbuscular mycorrhiza infected lower order roots without chitin sheaths also decayed slower (Fan et al. 2010). Therefore, these three hypotheses might not be solely attributed to control root litter decomposition, but affect root decomposition processes in combination with other root traits, climate, and soil conditions (Lin et al. 2011; See et al. 2019), more work is needed to disentangle the interactive effects of litter C, N, mycorrhizal infection, and other controls of root decomposition. Furthermore, root morphological properties are also probably linked to the differences in root decomposition (Makita et al. 2015). The morphological properties such as specific root length (SRL) can potentially affect the accessibility of substrates to decomposer (Swift et al. 1979). The decay of different-order roots is likely to occur through different contact area in the litter–soil and thus different microbial degradation activity (Chen et al. 2021). To consider both initial chemical and morphological root traits would help us better understand the underlying mechanisms of litter decomposition.

The smallest distal roots with rapid turnover rate but slow decomposition may provide the primary contribution to soil C and N accumulation in terrestrial ecosystems (Silver and Miya 2001; Fan et al. 2010). This effect of ephemeral roots on changing soil C pool would be profound under the global change (Davidson and Janssens 2006). Although the release of C and N at different root decomposing stages has drawn increasing attentions (Xiong et al. 2013; Zhuang et al. 2018), very few studies have so far involved the changes of soil C and N during root decomposition associated with root orders (Luo et al. 2016; Jacobs et al. 2018). Here, we measured root decomposition rate, the key root chemical and morphological trait parameters across four root branch order classes and the variations in soil C and N content below root litterbags for *Castanopsis fargesii* and *Schima*

*superba*, two species dominated early and late successional stages, respectively, in an evergreen broad-leaved forest (EBLF) in Eastern China. To enhance our understanding of fine-root decomposition and its influence on the nutrient processes in forest ecosystems, we attempted (1) to test whether slow decomposition of lower order roots would hold for these two subtropical key tree species; (2) to explore the relationships between root litter decomposition rates and their initial traits to the degree our data would allow; (3) to examine how C and N content vary in soil below the litterbag with different order roots decomposition.

## Methods

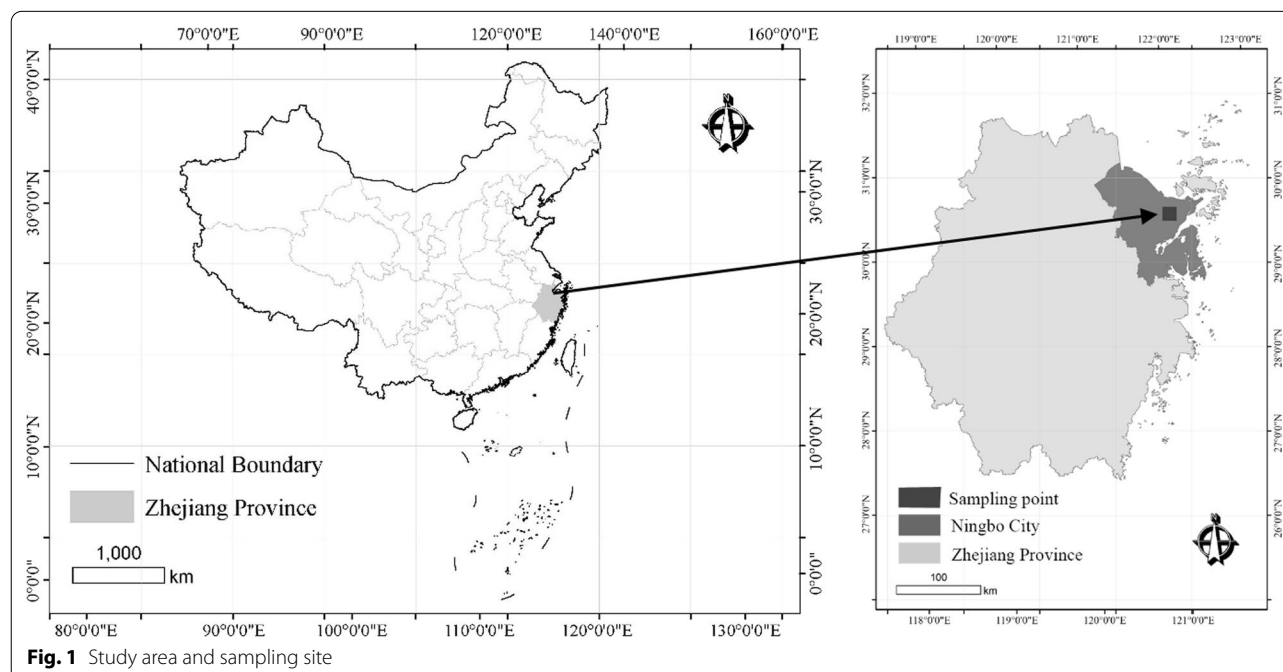
### Study site description

We collected root litter and soils at Tiantong National Forest Park (29°41'–50'N, 121°36'–52'E) located in Siming Mountain, Zhejiang Province, eastern coast of China (Fig. 1). The forest park has an area of 667 ha with an elevation ranging from 50 to 650 m. The study area has a typical monsoon climate with an annual mean temperature of 16.2 °C. The warmest month is July with a mean temperature of 28.1 °C, and the coldest is January with a mean temperature of 4.2 °C. The mean annual precipitation is 1374.7 mm, most of which distributed in May–August (Yan et al. 2007). The soils are belonged to Plinthosols according to WRB-based soil classification (Nachtergaele et al. 2000).

Evergreen broad-leaved forest (EBLF) is the typical ecosystem on Siming Mountain. The current vegetation is secondary forest recovering after the disturbance of the original forest. The mature forest around the famous Buddhist Tiantong Temple (>1600 years) in the center of the park has developed into climax monsoon EBLF dominated by *C. fargesii*. We chose *C. fargesii* in the climax EBLF stand and *S. superba* in the sub-climax EBLF stand for this study. The two stands, neighboring each other, had the same original vegetation in the history and the soils were developed from the same quartzitic parent material (Yan et al. 2006). The climax EBLF had a 150-year stand age with a canopy height of 25 m, while the sub-climax EBLF were rehabilitated 90 years ago and had established a canopy 20 m in height (Yan et al. 2009). The two species were selected, because they are both key species in natural forests of eastern China and *S. superba* is a common associate of *C. fargesii*. Soil properties and characteristics of litter and fine-root biomass in the *C. fargesii* and *S. superba* forest were demonstrated in Table 1 (Guo et al. 2011).

### Root litter and soils collection

Root sampling was conducted on July 24th of 2013, approximately when the belowground growth peaked. In each forest type, three 30 m × 30 m plots were established on an approximately 25° slope of south-east facing, with an elevation ranging from 150 to 220 m. In each plot, we chose three trees with similar height and aboveground size for root collections. Under every tree,



**Table 1** Soil properties as well as litter and fine-root biomass of the *C. fargesii* and *S. superba* stands

	<i>Castanopsis fargesii</i>	<i>Schima superba</i>
Community types	Climax evergreen broad-leaved forest	Sub-climax evergreen broad-leaved forest
Dominant tree species	<i>C. fargesii</i> <i>S. superba</i> <i>Liquidambar formosana</i>	<i>S. superba</i> <i>Lithocarpus glabra</i> <i>Pinus massoniana</i>
TOC (g kg <sup>-1</sup> )	56.7 ± 3.3 <sup>a</sup>	35.0 ± 1.3 <sup>b</sup>
TN (g kg <sup>-1</sup> )	5.2 ± 0.1 <sup>a</sup>	2.6 ± 0.1 <sup>b</sup>
pH	3.9 ± 0.1 <sup>b</sup>	4.7 ± 0.4 <sup>a</sup>
Bulk density (g cm <sup>-3</sup> )	1.07 ± 0.06 <sup>b</sup>	1.30 ± 0.04 <sup>a</sup>
Saturation moisture content (%)	37.8 ± 0.3 <sup>a</sup>	30.5 ± 1.1 <sup>b</sup>
Soil temperature (°C)	13.3 ± 0.1 <sup>a</sup>	13.4 ± 0.2 <sup>a</sup>
Soil C/N ratio	11.0 ± 0.6 <sup>b</sup>	13.3 ± 0.2 <sup>a</sup>
Soil MBC (mg kg <sup>-1</sup> )	296.5 ± 20.4 <sup>a</sup>	271.9 ± 16.8 <sup>a</sup>
Litter biomass (kg m <sup>-2</sup> )	1.3 ± 0.1 <sup>a</sup>	1.6 ± 0.2 <sup>a</sup>
Fine-root biomass (t hm <sup>-2</sup> )	3.1 ± 0.2 <sup>a</sup>	2.8 ± 0.04 <sup>a</sup>

Different letters for each property indicate significant differences between these two stands ( $P \leq 0.05$ )

TOC total organic carbon, TN total nitrogen, C carbon, N nitrogen, MBC microbial biomass carbon

we first removed the surface soil until we saw the axial root. Then we continued to clear the surface soil along large branches till the terminal segments. Finally, we took a soil block of about 30 cm (L) × 20 cm (W) × 10 cm (H) with the axial root in the center (Xiong et al. 2013; Sun et al. 2015). We transported the soil blocks to laboratory to separate roots by branch order carefully. There were three blocks of soil were taken for each tree. Thus, a total of 27 soil blocks were collected for each species. Soils were air-dried, sieved to 2 mm to remove rocks and roots, and then homogenized. To achieve roots of the five orders, we took a two-step process. The first step was to separate the whole segments of root system from the soil. It was processed carefully to maintain the intactness of root branching systems. Then each entire root branch with at least five-order lateral roots was gently washed with deionized water (at 1 °C) to clear the attached soil particles. The second step was to separate the roots by order. After rinse, the roots were kept in deionized (at 1 °C) water and dissected by branch orders. According to the classic “developmental” approach, distal roots were classified as first order. The first-order branches from them were defined as second order, and so on (Berntson 1997; Pregitzer et al. 2002). The first two orders of roots generally lack the secondary tissue and have similar patterns of decomposition that are distinct from the third-order roots (Guo et al. 2008b). Besides, in a preliminary examination we found that there were no significant differences in C/N ratio and N concentrations of root litters between the first and second orders in the two species. Therefore, we separated fine roots into four-order classes:

RO1–2 included the first two orders of roots; RO3, RO4 and RO5 were comprised of the third, fourth and fifth root segments, respectively. A total of 24 specimens (2 species × 4 root-order classes per species × 3 replicates per root-order class) were obtained.

#### Measurements of root morphological traits

Due to a large number of root individual segments of each specimen, we randomly selected 100 roots of RO1–2 and 20 roots of RO3, RO4 and RO5 in each specimen for the assessment of root diameter and length. The individual roots selected in the same specimen were laid in a tiny clear acrylic tray with enough deionized water on the bottom. It was placed in a dual light source scanner (ScanMakeri800 Plus, Microtek Science and Technology Inc., China) to get the root image, which was analyzed by software WinRHIZO TRON (Regent Instruments, Canada). The diameter of an individual root and its length was assessed. The scanned roots of each specimen were dried (70 °C for 48 h) to constant for dry mass measurement and subsamples were placed in muffle furnace (550 °C for 4 h) for ash determination. Then specific root length (SRL, m g<sup>-1</sup>) was calculated from the cumulative fresh length and ash-corrected mass for each specimen.

#### Root litterbag incubation and harvest

We conducted a 540-day incubation experiment using root litter and soils collected from the field plots. The roots were cut into similar length and the soil was a homogenous mix from the two sites. We took five

samples from each specimen, each with an accurate dry weight of  $4.0000 \pm 0.0010$  g roots, and sealed in five litterbags. The litterbags were made of nylon with 0.1 mm mesh screen and a size of 4 cm in length and 4 cm in width. We used ceramic pots with a height of 10 cm and 8 cm in diameter as the incubation vessel. Each pot was filled with 150 g soils and one litterbag was placed on the top with a coverage of another 50 g soils. Water was added to reach 60% water holding capacity. All pots were weighted weekly to add deionized water for maintaining soil moisture constantly. The incubation was conducted in an artificial climate box (HPG-280 HX, Harbin Donglian Electronic Technology Development Co., Ltd, China) under dark conditions at a temperature of 25 °C and a relative humidity of 80%. In 30, 90, 180, 360 and 540 days after incubation, three litterbags were harvested for each root-order class of both species, together with soil samples below the litterbags.

#### Root and soil chemical analyses

After harvest, root litter was rinsed with deionized water and dried at 65 °C to get the weight. Soil samples below the litterbag from each pot were sieved and air-dried. Root litter and soil samples were then separately ground to homogeneous fine powders using a MM 200 steel ball mill (Retsch GmbH, Haan, Germany). They were packed in tin cups and combusted in an elemental analyzer (Vario EL cube, Elementar, German) for total C and N analyses. Root C-fraction concentrations comprising extractive fractions (EF, more degradable carbon including nonpolar compounds, such as fatty acids and lipids as well as polar compounds, such as sugars and phenolics), acid-soluble fraction (ASF, moderately decomposable compounds including polymer carbohydrates, hemicellulose and holocellulose) and acid-insoluble fraction (AIF, highly recalcitrant compounds including lignin and acid-insoluble aromatic compounds) were measured using forest products serial digestion technique (Ryan et al. 1990). All root chemistry values are expressed on an ash-free, dry mass basis. Soil dissolved organic carbon (DOC) was extracted by adding 2 mol L<sup>-1</sup> potassium chloride to subsample of 20 g homogenized soil, and agitating on an orbital shaker at 200 rpm for 1 h. The filtrate was analyzed using a TOC analyzer (Shimadzu, TOC-5000A, Japan). An additional set of 3 replicates of each root specimen was taken for initial chemistry analyses. The soil C, N and DOC content of the mixed homogenous soil before incubation was also measured with 3 replicates.

#### Data analysis

Mean values of all chemical and morphological parameters per order class and species were calculated. All data were satisfied the assumption of normality except

SRL. Therefore, SRL was log<sub>10</sub>-transformed to improve the normality. Two-way analysis of variance (ANOVA) was employed to identify the effects on root indices with species and root order as the fixed effects. For every parameter, Fisher's protected LSD test was applied to the pairwise comparisons among orders. Analysis of covariance (ANCOVA) was conducted to compare the differences in remaining percentage of initial root mass among order classes for each species with decomposition time as the covariate. Differences in root C and N contents, soil C, N, DOC concentration and C/N ratio across all order classes over decay time were also analyzed by ANCOVA. A negative exponential decay model was used to obtain the decomposition constant ( $k$ ) as described by Olson (1963). The model was  $\ln(M_t/M_0) = -kt$ , where  $M_t$  is the litter mass at time  $t$  and  $M_0$  is the initial litter mass. Structural equation modeling (SEM) was used to analyze the hypothetical pathways of species and root-order effects on root mass remaining at the end of the 540-day incubation through contents of EF, N, and AIF, as well as C/N ratio, diameter, and SRL. Data were fitted to the models applying the maximum likelihood estimation method. Fitness of the model was evaluated using  $\chi^2$  tests, Akaike Information Criteria (AIC), and root square mean errors of approximation (RMSEA) (Grace 2006). Statistical analyses were performed using SPSS version 11.5 (SPSS Inc., IBM, Armonk, NY, USA). In all cases, a  $P$  value  $\leq 0.05$  was considered to be significant. The SEM was performed using AMOS 22.0 (Amos Development, Spring House, Pennsylvania, USA).

## Results

### Initial root chemical and morphological properties

There were significant impacts of order ( $P < 0.001$ , Table 2) and species ( $P < 0.001$ , Table 2) on root C concentration. Pattern of root C concentrations across orders was characterized by marked increase from RO1–2 to RO3, then leveling off for RO4 and RO5 (Table 2). The C concentration in *C. fargesii* roots ( $414.4\text{--}477.9$  mg g<sup>-1</sup>) was lower than *S. superba* ( $453.0\text{--}499.7$  mg g<sup>-1</sup>) within each root order (Table 2).

The order ( $P < 0.001$ , Table 2) and species ( $P < 0.001$ , Table 2) also had significant effects on root N concentration. For both species N in root tissues decreased by increasing root order (Table 2). Roots contained  $17.2 \pm 2.4$  mg N g<sup>-1</sup> for RO1–2 roots and decreased to  $11.5 \pm 1.2$  mg N g<sup>-1</sup> for RO5 roots for *C. fargesii* (Table 2). A similar trend was also for *S. superba* in root N concentration, decreasing from  $12.0 \pm 0.9$  to  $7.1 \pm 0.8$  mg N g<sup>-1</sup> with ascending order (Table 2).

The EF and AIF concentrations were significantly affected by species and root order, whereas there were no significant differences in root ASF content of the



**Table 2** Initial tissue chemistry and morphology of different-order roots at the beginning of litterbag experiment for the two subtropical tree species (mean with SE in parentheses,  $n=3$ )

Initial root	Initial chemistry							Initial morphology	
	C (mg g <sup>-1</sup> )	N (mg g <sup>-1</sup> )	EF (mg g <sup>-1</sup> )	ASF (mg g <sup>-1</sup> )	AIF (mg g <sup>-1</sup> )	C/N ratio	AIF/N ratio	SRL (m g <sup>-1</sup> )	Diameter (mm)
<i>C. fargesii</i>									
RO1–2	414.4 <sup>b</sup> (16.5)	17.2 <sup>a</sup> (2.4)	331.2 <sup>ab</sup> (35.6)	217.7 <sup>b</sup> (47.9)	370.5 <sup>a</sup> (63.3)	24.5 <sup>b</sup> (3.8)	21.6 <sup>a</sup> (3.5)	57.49 <sup>a</sup> (14.43)	0.23 <sup>c</sup> (0.03)
RO3	473.0 <sup>a</sup> (10.3)	13.1 <sup>b</sup> (0.8)	289.3 <sup>b</sup> (45.5)	213.1 <sup>b</sup> (37.5)	244.7 <sup>b</sup> (56.1)	36.2 <sup>a</sup> (3.0)	18.7 <sup>ab</sup> (4.2)	8.78 <sup>b</sup> (2.02)	0.52 <sup>c</sup> (0.04)
RO4	476.9 <sup>a</sup> (12.8)	11.8 <sup>b</sup> (0.6)b	347.9 <sup>ab</sup> (40.2)	205.0 <sup>b</sup> (16.4)	170.4 <sup>bc</sup> (52.3)	40.4 <sup>a</sup> (0.8)	14.3 <sup>ab</sup> (3.8)	2.95 <sup>b</sup> (0.62)	0.89 <sup>b</sup> (0.03)
RO5	477.9 <sup>a</sup> (16.1)	11.5 <sup>b</sup> (1.2)	376.5 <sup>a</sup> (60.2)	294.8 <sup>a</sup> (41.7)	131.5 <sup>c</sup> (59.3)	41.9 <sup>a</sup> (5.3)	11.5 <sup>b</sup> (5.1)	0.90 <sup>b</sup> (0.21)	1.71 <sup>a</sup> (0.33)
<i>S. superba</i>									
RO1–2	453.0 <sup>b</sup> (16.3)	12.0 <sup>a</sup> (0.9)	296.2 <sup>a</sup> (31.2)	271.9 <sup>a</sup> (39.9)	414.4 <sup>a</sup> (41.6)	38.0 <sup>c</sup> (1.3)	34.8 <sup>a</sup> (5.3)	66.14 <sup>a</sup> (10.41)	0.23 <sup>d</sup> (0.03)
RO3	499.7 <sup>a</sup> (5.5)	9.2 <sup>b</sup> (1.0)	206.0 <sup>b</sup> (40.0)	245.2 <sup>a</sup> (58.6)	285.9 <sup>ab</sup> (14.9)	55.0 <sup>b</sup> (5.1)	31.6 <sup>a</sup> (4.8)	17.31 <sup>b</sup> (2.05)	0.39 <sup>c</sup> (0.02)
RO4	496.7 <sup>a</sup> (13.00)	7.6 <sup>b</sup> (1.3)	273.8 <sup>ab</sup> (28.0)	268.5 <sup>a</sup> (12.2)	275.8 <sup>b</sup> (96.1)	66.1 <sup>ab</sup> (9.1)	38.2 <sup>a</sup> (19.1)	5.68 <sup>c</sup> (0.75)	0.58 <sup>b</sup> (0.04)
RO5	494.6 <sup>a</sup> (9.3)	7.1 <sup>b</sup> (0.8)	305.6 <sup>a</sup> (45.7)	252.2 <sup>a</sup> (16.7)	199.4 <sup>b</sup> (100.9)	70.3 <sup>a</sup> (6.7)	29.3 <sup>a</sup> (17.0)	1.84 <sup>c</sup> (0.28)	1.08 <sup>a</sup> (0.15)
<i>P</i> values									
Order	<0.001	<0.001	0.010	0.225	<0.001	<0.001	0.571	<0.001	<0.001
Species	<0.001	<0.001	0.001	0.098	0.029	<0.001	0.001	<0.001	<0.001
Order × species	0.494	0.818	0.766	0.098	0.822	0.084	0.745	0.056	0.004

Means with different letters are significantly different among different root-order classes ( $P \leq 0.05$ )

C—carbon; N—nitrogen; EF—extractive fraction; ASF—acid-soluble fraction; AIF—acid-insoluble fraction; SRL—specific root length. RO1–2 refers to the combination of the 1st- and 2nd-order roots, RO3, RO4 and RO5 refer to the 3rd-, 4th- and 5th-order roots, respectively

Probabilities show two-way ANOVA of chemical and morphological properties. The values of *P* lower than 0.05 present in bold font

two species among the four root-order classes (Table 2). Moreover, only tree species had significant effects on AIF/N ratio ( $P=0.001$ , Table 2). *C. fargesii* roots always had a lower AIF/N ratio with an average of 16.5, which was 33.5 for *S. superba* (Table 2).

Both species and order had significant impacts on diameter and SRL (Table 2). Across the root orders, the variations in root diameter ranged from a minimum of  $0.23 \pm 0.03$  mm to a maximum of  $1.71 \pm 0.33$  mm in *C. fargesii* and from  $0.23 \pm 0.03$  to  $1.08 \pm 0.15$  mm in *S. superba*, respectively (Table 2). *C. fargesii* roots had significantly larger diameter and smaller SRL than *S. superba* ( $P < 0.001$ , Table 2).

#### Root mass loss and N dynamics during decomposition

Over the 540-day period, mass remaining was the highest in RO1–2 and the lowest in RO5 roots for *C. fargesii* ( $P < 0.001$ , Fig. 2A), while the difference between root orders was not significant for *S. superba* ( $P=0.241$ , Fig. 2B). For both species, the highest *k* was observed in RO5 roots with decreasing *k* in lower root order (Table 3). Comparison between the two species, *C. fargesii* roots decomposed faster than *S. superba* within each root order (Fig. 2; Table 3).

C loss from *C. fargesii* root was 7.9% in RO1–2, 16.7% in RO3, 8.3% in RO4, and 9.6% in RO5 (Fig. 3A). For *S.*

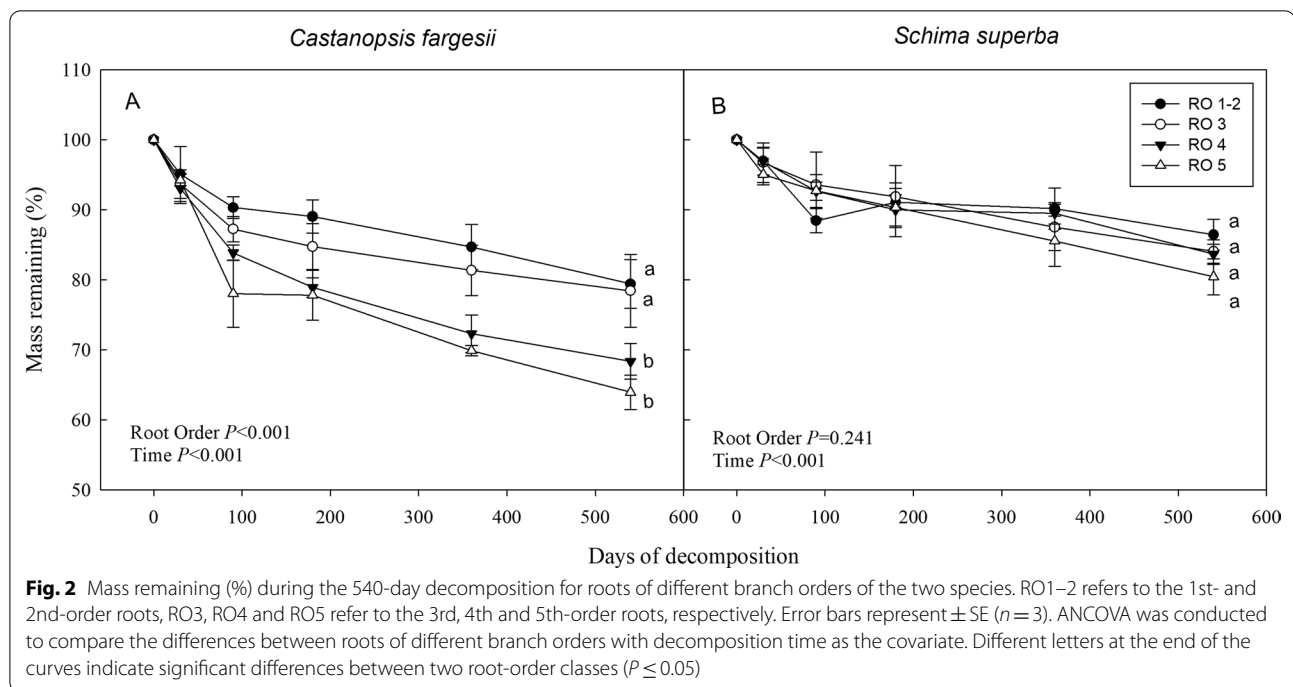
*superba* root these values were 6.0%, 10.9%, 14.0%, and 8.9%, respectively (Fig. 3B). Contrary to root mass and root C, root N content declined more rapidly in RO1–2 roots than the other three orders of roots, especially in the earlier stages of decomposition (Fig. 3C, D). After 540 days of decay, RO1–2 roots lost 22.7% and 17.3% of initial N for *C. fargesii* and *S. superba*, respectively.

#### Relationship between remaining mass with root litter traits

The SEM explained 84.7% of the variation in root mass remaining percentage, 30.9%, 91.7%, 87.8% 72.0%, 72.9% and 98.7% of the variation in root EF content, C/N ratio, N content, AIF content, root diameter, and SRL, respectively (Fig. 4). Species had direct effects on these root properties. Moreover, root order also directly affected root traits except for EF content and C/N ratio. Root order had positive direct pathways to root diameter, but negative pathways to root N, AIF content and SRL. Overall, the SEM showed that root C/N ratio and SRL positively related to the final root remaining mass percentage, while root diameter negatively correlated with it.

#### Soil C and N content changes during decomposition

Over the 540-day period, no significant differences of soil C, N, DOC content and C/N ratio below the litterbags with roots of the four-order class were found in the



**Table 3** Root mass remaining (%), decomposition constants ( $k$ , year<sup>-1</sup>) and the associated  $R^2$  values of the four root-order classes obtained by fitting a single exponential decay model for the two subtropical tree species (mean with SE in parentheses,  $n=3$ )

Root litter	<i>C. fargesii</i>			<i>S. superba</i>		
	Mass remaining (%)	$k$ (year <sup>-1</sup> )	$R^2$	Mass remaining (%)	$k$ (year <sup>-1</sup> )	$R^2$
RO1–2	79.4 (3.5) <sup>a</sup>	0.17	0.81	86.4 (2.2) <sup>a</sup>	0.10	0.77
RO3	78.4 (5.2) <sup>a</sup>	0.19	0.58	84.1 (1.6) <sup>ab</sup>	0.12	0.88
RO4	68.4 (2.5) <sup>b</sup>	0.30	0.76	83.7 (1.5) <sup>ab</sup>	0.12	0.77
RO5	63.9 (2.4) <sup>b</sup>	0.34	0.74	80.4 (2.6) <sup>b</sup>	0.15	0.88

Mean with different letters are significantly different between two root-order classes ( $P \leq 0.05$ )

RO1–2 refers to the combination of the 1st- and 2nd-order roots, RO3, RO4 and RO5 refer to the 3rd-, 4th- and 5th-order roots, respectively

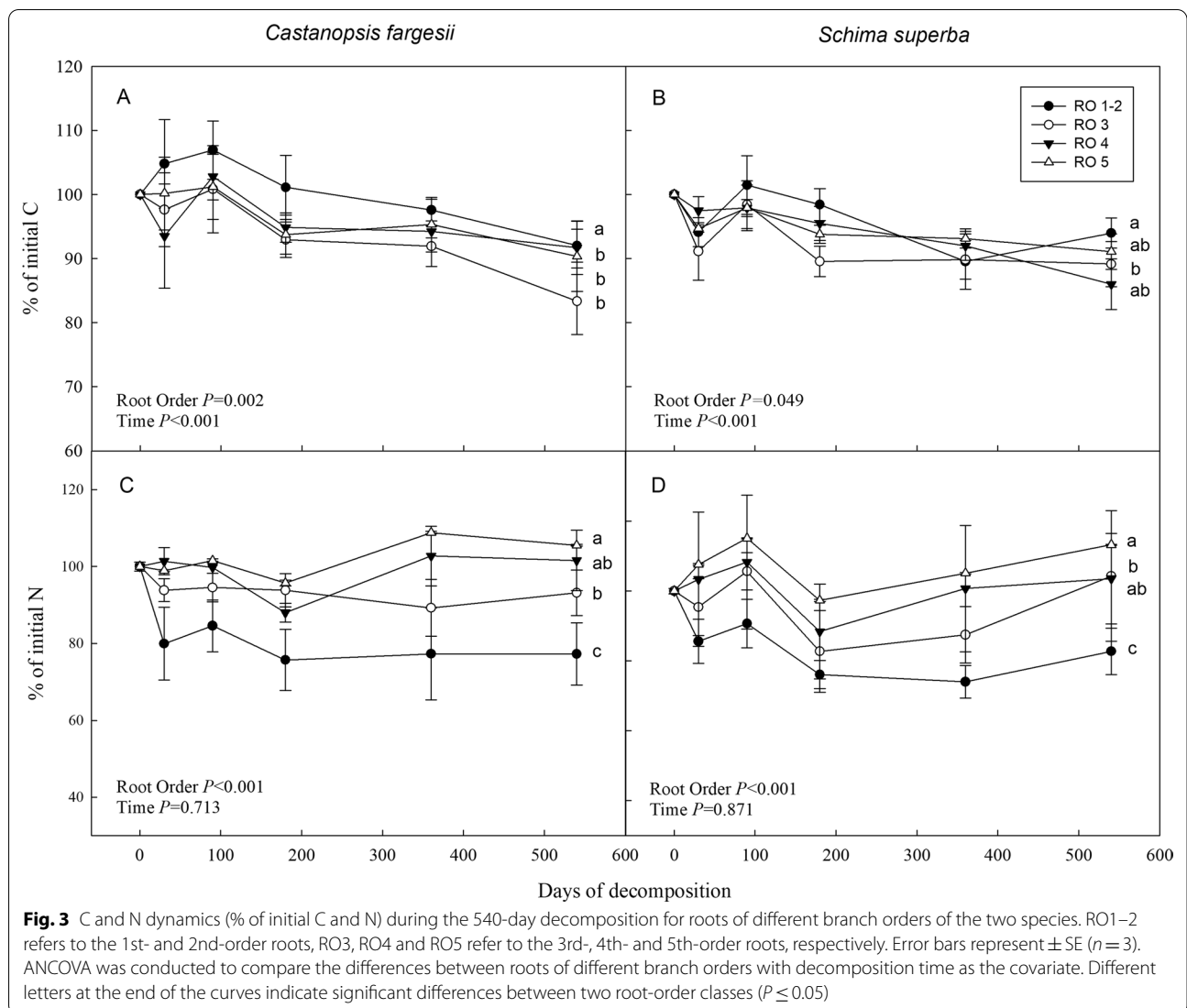
two species (Fig. 5). The variations of soil C below the litterbags with the four-order roots had similar patterns. Total soil C content decreased gradually over the whole decaying period with a minor increment around day 180 (Fig. 5A, B). After 540 days of decay, the soil C content declined 8.53% and 7.63% on average below the litterbag with *C. fargesii* and *S. superba* roots, respectively. The total soil N content increased rapidly during the initial 30 days of the observation period and then declined till the 360th day, but appeared increasing once again at the end of the experiment for both species (Fig. 5C, D). Due to the changes of soil C and N content, soil C/N ratio experienced a rapid declining in the initial 30-day period, then a gradual increasing till the 360th day, and a decreasing again in the last period of the 540-day

incubation (Fig. 5E, F). Soil DOC also declined quickly at the first 30-day decomposing stage, but increased around day 90 and then declined gradually with a slight increase at the time of day 360 in soils with litter from all the root-order classes of both species (Fig. 5G, H).

## Discussion

### Different roles of species and root order in fine-root decomposition

Root decomposition is an important ecological process in regulating soil C and nutrient cycling; meanwhile, it is also a complex process that the heterogeneity in decay rate strongly related to root traits, soil properties, decomposer and climate (Silver and Miya 2001; Smith et al. 2014). Root branch order is considered as a crucial index

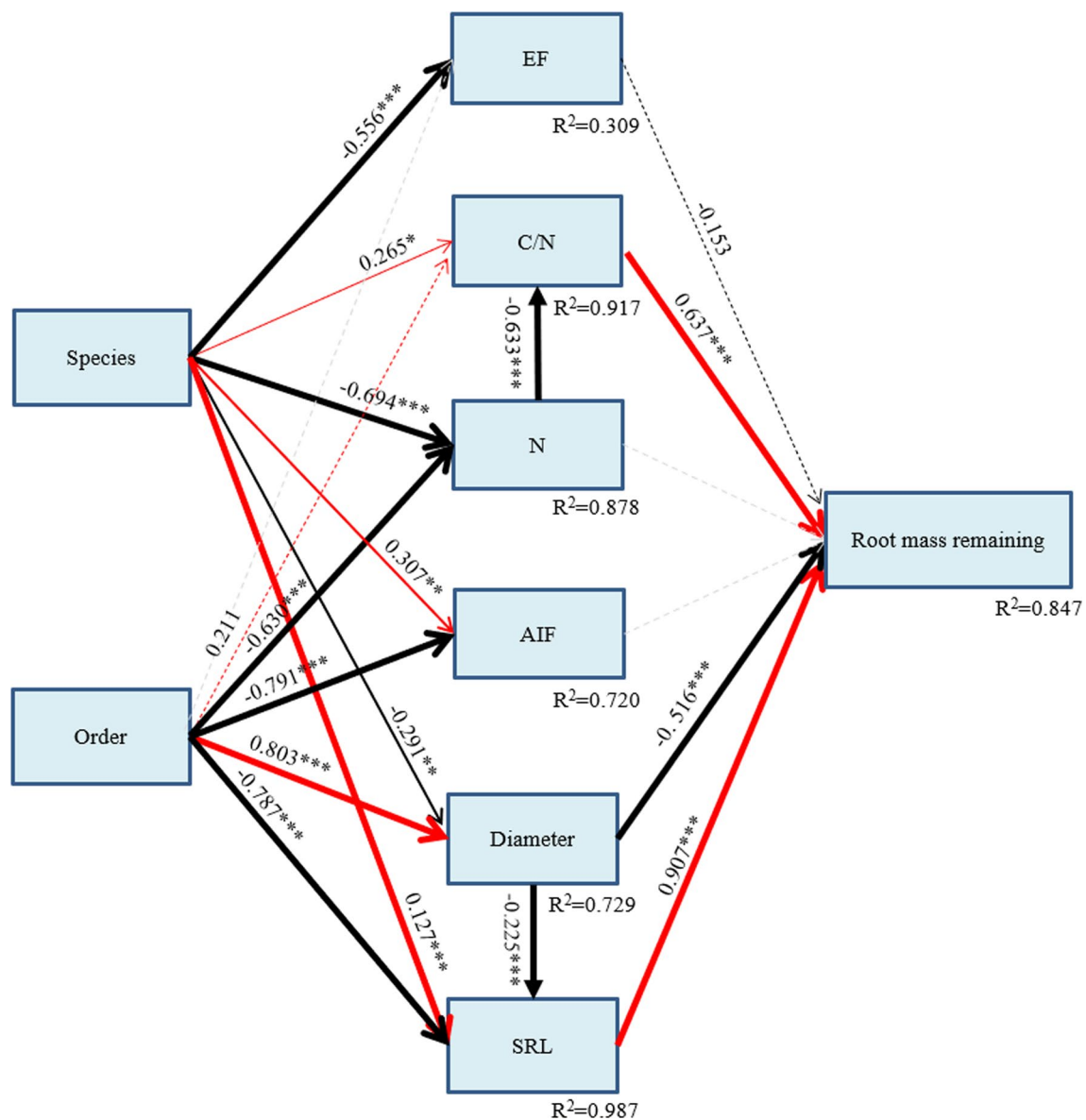


to identify functional heterogeneity of tree fine-root systems (Guo et al. 2008a, b). Here we aimed to examine that whether root branch orders would affect root decomposition processes of two dominant subtropical species of eastern China. Our study found that root order had different effects on the decomposition rate in these two species. Lower order roots decayed slower than higher order roots for *C. fargesii* ( $P<0.001$ , Fig. 2A), but it's not true for *S. superba* ( $P=0.241$ , Fig. 2B). So far, only few studies assessed root orders in relation to their decomposition rate, suggesting that first- and second-order roots decomposed more slowly than higher order roots in temperate forest (Fan and Guo 2010; Goebel et al. 2011). However, this pattern was partly supported in case of our study, which is in consistent with the results of Xiong et al. (2013), in which two out of four subtropical trees studied mass remaining did not differ significantly with

order classes. These indicated that the pattern of slower decomposition rates in lower order than higher order roots might not be significantly held for all subtropical trees. To validate the commonality of this pattern more studies on various species are needed, if it exists.

Moreover, in the current case, root litter of *C. fargesii* tended to decompose more rapidly compared to those of *S. superba* roots (Fig. 4; Table 3). Correspondingly, *C. fargesii* was collected in the climax EBLF stand and *S. superba* was from the sub-climax EBLF stand in this study. The two stands, neighboring each other, had the same original vegetation in the history and the soils were developed from the same quartzitic parent material (Yan et al. 2006). This may indicate that dominant tree species shifted during forest succession, which in turn may greatly impact soil C and nutrient retention through root decomposition dynamics.

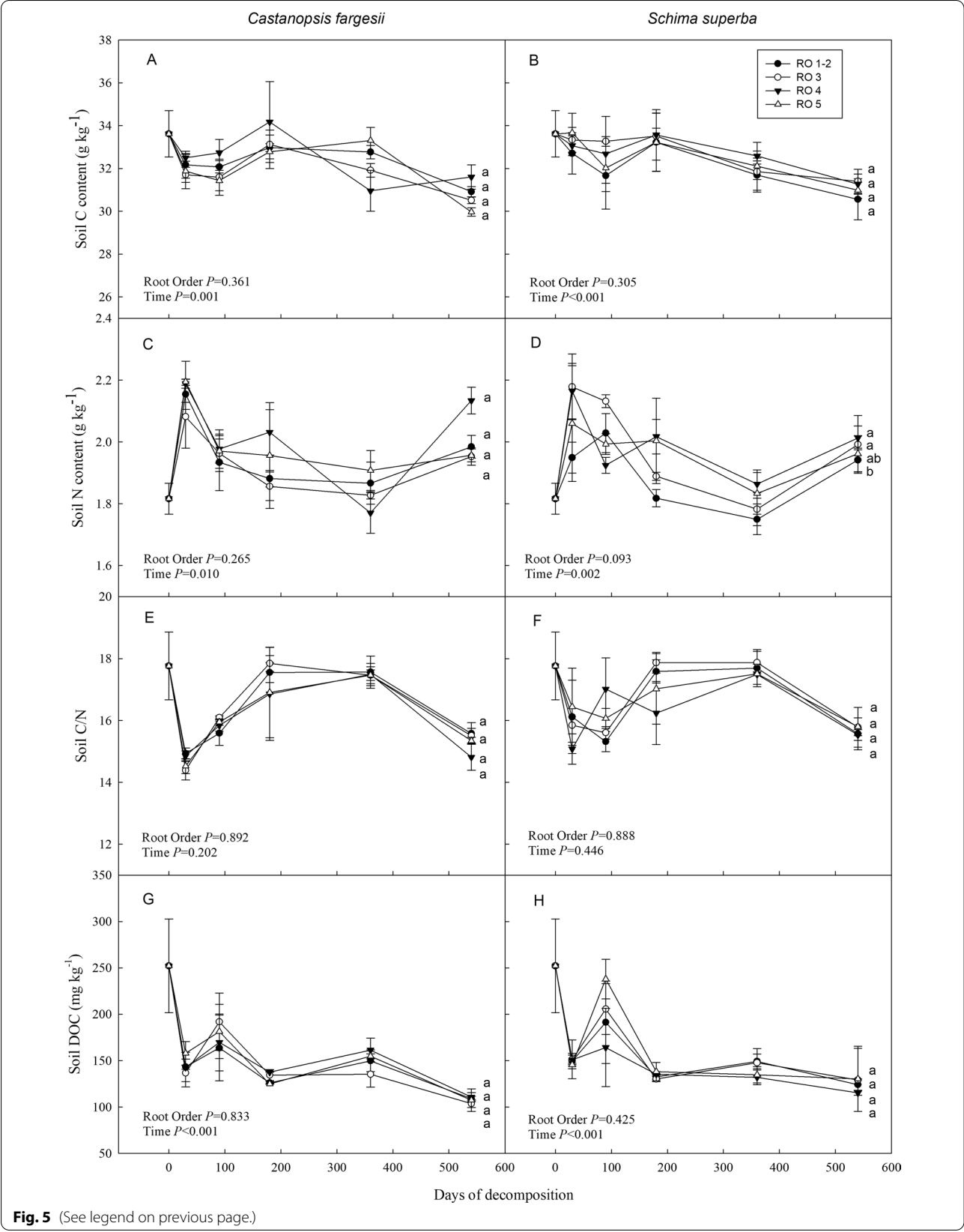




**Fig. 4** Structural equation model of species and root-order effects on root decomposition. Relationships between species, root order and root chemical and morphological traits (EF, C/N, N, AIF, SRL and Diameter), and root mass remaining percentage. The final model fit the data well:  $\chi^2 = 18.321$ ,  $df = 18$ ,  $P = 0.435$ , Akaike Information Criteria (AIC) = 90.321, Root mean square error of approximation (RMSEA) = 0.028. Numbers adjacent to the arrows are standardized regression weights (\* $P \leq 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). Red arrows represent positive relationships and black arrows represent negative relationships, respectively. The width of the arrows indicates the strength of the relationships. Red and black dashed arrows representing positive and negative nonsignificant paths are kept. Gray dashed arrows indicate nonsignificant paths removed to improve model fits.  $R^2$  values below each variable indicates the proportions of variation explained by the model

(See figure on next page.)

**Fig. 5** Soil C, N, DOC concentrations and C/N during the 540-day root decomposition of different branch orders of the two species. RO1–2 refers to the 1st- and 2nd-order roots, RO3, RO4 and RO5 refer to the 3rd-, 4th- and 5th-order roots, respectively. Error bars represent  $\pm SE$  ( $n = 3$ ). ANCOVA was conducted to compare the differences between roots of different branch orders with decomposition time as the covariate. Different letters at the end of the curves indicate significant differences between two root-order classes ( $P \leq 0.05$ )



**Fig. 5** (See legend on previous page.)

### Underlying mechanisms in controlling root decomposition

In general, root litter quality is considered as one of the most important factors controlling decomposition rate (Silver and Miya 2001; Zhang and Wang 2015). Our SEM demonstrated that species directly influenced initial root C/N ratio as well as the concentration of N, EF and AIF. Moreover, species had indirectly positive effects on root mass remaining through C/N ratio (Fig. 4). Root order had direct relation with initial root N and AIF concentration, but there were no significant pathways to root mass remaining through these two tissue components (Fig. 4). Irrespective of tree species and root order, C/N ratio might be the best predictors of root decomposition among the measured chemical traits in the current case. The importance of C/N ratio has been extensively recognized to characterize litter chemical quality (Silver and Miya 2001; Giweta 2020), which was also shown in our SEM result (Fig. 4). However, the positive relationship between C/N ratio and root mass remaining for these two species contrasted with the negative relationship between C/N ratio and root mass remaining among root orders within the same species, suggesting that C/N ratio may not merely depend on the proportions of C and N, but on the C fractions related to N. Our data showed that *C. fargesii* roots had lower AIF decomposed faster than *S. superba* roots, and consistently the higher order roots of *C. fargesii* with lower AIF also decomposed faster than the lower order roots (Table 2; Fig. 2). In addition, the correlation coefficients with mass remaining percentage were higher in AIF/N ratio ( $r=0.765$ ,  $P<0.001$ , Additional file 1: Table S1) than AIF ( $r=0.642$ ,  $P=0.001$ , Additional file 1: Table S1) for the pooled data. These results suggest that it is not C content alone, but the linkage of C component and N determines chemical quality for litter decomposition.

Moreover, we found that the significant differences in mass loss among order classes for *C. fargesii* roots (Fig. 2A), corresponding with their wide differences in AIF/N ratio (Table 2). By contrast, decomposition rate did not differ significantly across root orders in *S. superba* (Fig. 2B), in consistence with the similar value of AIF/N ratio across root-order classes (Table 2). This may also suggest that the influence of root chemical quality on root decomposition is complex and depends on C and N interactions (Zhang et al. 2008). Given that lower order roots have very low lignin content base on their anatomy (Guo et al. 2008b), the higher content of AIF may be primarily composed of recalcitrant compounds, such as defensive secondary metabolites (Seastedt and Murray 2008), which supply less energy for decomposer (Hättenschwiler and Jørgensen 2010). Microbial decomposition is expected to slow down due to progressive labile C limitation (Berg and McClaugherty 2014), and such

limitation would be more severe under higher N or lower C/N ratio in lower order roots. Thereby, C quality linked to N concentration may be the key controller of decomposition among different orders of roots for this subtropical species.

In consistence with recent studies representing negative effect of these acid-insoluble fractions on root decomposed rate (Sun et al. 2013; Makita et al. 2015), AIF significantly positively correlated with mass remaining percentage across root-order classes in pooled data ( $r=0.642$ ,  $P=0.001$ , Additional file 1: Table S1), while EF significantly negatively correlated with mass remaining percentage ( $r=0.642$ ,  $P=0.001$ , Additional file 1: Table S1). This further implied the regulating roles of root litter C components in the decomposition. The disappearance of significant paths from EF and AIF to mass remaining in SEM (Fig. 4) was mainly due to the contrary effects between root order and species. Despite of similar patterns in EF, AIF, and mass remaining across root orders in these two species, there were large differences in the ranges of these parameters between the two trees. Furthermore, this study may be still in an early stage of litter decomposition (Harmon et al. 2009), when labile C is the main energy supplement for microbes to influence root decaying rates. The reported studies showed that the influence of higher AIF concentrations always appeared more than 2 years after incubation, when the degradable C might be exhausted in the later stage of decomposition (Parton et al. 2007; Xiong et al. 2013). Species, root-order class, and decaying period should be considered to understand the relationship between root decomposition rates and their initial chemical traits because of the different underlying mechanisms.

Moreover, root morphological trait rather than root chemistry might be more likely to make root decay rate different among root-order classes in current study, as the summed effect of indirect pathways to root mass remaining through “Diameter” and “SRL” was much higher than that through the four chemical traits (Fig. 4). This is in according with the notion that root litter–soil contact might be one of the important factors to influence the rate of decomposition (Berntson 1997; Chen et al. 2021). High degree of root–soil contact could improve the accessibility of root tissue to decomposers and may facilitate microbial catabolism by modifying litter–soil temperature and moisture conditions. However, we found a positive relationship between SRL and root remaining mass, which contrasted with the previous studies (Smith et al. 2014). This might be explained by the tendency of being easily compacted for the lower order roots with higher SRL because of their thinner diameter, leading to poor contact with soil (Chen et al. 2021). Another possible explanation is that the thinner lower order roots were

more likely to attract adhesive water to create an anaerobically condition around them, which inhibited microbial degradation activity. The recent evidence showed that root remaining mass was negatively correlated to the initial specific root area (SRA), but positively related to the remaining SRA (Makita et al. 2015). Hence, although root morphology might play a more important role in regulating fine-root decomposition rate, the initial and residual morphological properties should be considered to determine the process of degradation. Nonetheless, the direct mechanistic investigations of the control factors on root decomposition are still sparse, demanding more studies.

### Influences of root decomposition on soil C and N

Root litter decomposition represents a major sources of soil C accumulation in terrestrial ecosystems (Silver and Miya 2001; See et al. 2019). Traditional paradigm stated that slow decomposing litter contributed more to soil C storage than more rapidly decaying litter, because the recalcitrant fraction in litter resisting degradation would accumulate in soil (Lehmann and Kleber 2015; Poirier et al. 2018). We did not find evidence of clear differences in soil C and N content under the litterbags with different-order roots. This might be explained by a combination effect linking to root mass loss, root litter quality, and microbial activities (Fornara et al. 2020; Manzoni et al. 2021). Part of the disappeared litter is incorporated into soil in the formation of particulate organic matter, dissolved organic compounds, or microbial transformed litter compounds (Klotzbücher et al. 2016). The quantity of litter mass loss is not equal to litter-derived organic matter that is incorporated into soil through microorganisms (Liang et al. 2017; Manzoni et al. 2021). A recent synthesis showed that more slowly decomposing litters had a higher efficiency of C and N transfer to the soil compared to more rapidly decomposing litter (Zheng et al. 2021). This indicated that recalcitrant (i.e., lignin) fraction in litter did not directly stabilize in soil, but was also degraded by microorganisms (Soares and Rousk 2019). In addition, the litterbag method constrained the mixing of litter and soil and may cause difference in transferring and incorporating C and N into soil during decomposition (Ma et al. 2016; Chen et al. 2021). Although the standard litterbag methods isolated the direct contact between soil and roots and potentially depressed decomposition rate of lower order roots more than higher order roots (Chen et al. 2021), this study detected a likely trend that lower order roots containing less degradable carbon and more acid-insoluble components would decompose slowly, lost less root mass, but added more microbial-derived C to the soil, while higher order roots having more readily decomposable components and fewer recalcitrant compounds decomposed

fast, lost more root mass, but transferred less microbial-derived C to the soil (Figs. 3, 5). Therefore, more works are needed to accurately understand the belowground C cycle.

This study demonstrated a significant temporal variation of soil C and N content under root litterbag during the decaying (Fig. 5). For both species soil N content increased up to the initial 30 day and then decreased, with another increment at the end of the incubation (Fig. 5C, D). Litter N content was relatively stable over the whole decaying period except for an obvious decrease in RO1–2 of both species and an increase in RO5 in *S. superba* in the first 30–60 days (Fig. 3C, D). Together with a gradual declination of soil N content in most cases (Fig. 5C, D), it may indicate that the initial elevation of soil N content was not sustainable by labile N releasing in the early decaying stage or by the bonded N released from the recalcitrant fraction of litter in later stage (Parton et al. 2007). Other studies also provided evidences for the slow release of N from plant litter (Lindahl et al. 2007; Xiong et al. 2013; Chen et al. 2021) or a balance between rapid release from litter and incorporation of soil N into litter through fungal transfer (Zeller et al. 2000). The labile C input from root litter would stimulate the activity of microorganisms as N might be a limiting factor for microbial growth (Manzoni et al. 2021). Thus, the initial peak of soil N content may be partly due to strong microbial mineralization of N from recalcitrant organic matter to acquire N (Craine et al. 2007). In addition, the highest N content coincident with the lowest DOC content in the first 30 days may indicate a stimulation of soil copiotrophic microorganisms, and the subsequent oscillation of soil N and DOC content (Fig. 5G, H) may reflect a shift of soil microbial composition between copiotrophs and oligotrophs (Finn et al. 2015). The final decrease in DOC was consistent with results from a field experiment (Zhou et al. 2015), probably owing to a decrease in root litter mass, as the field experiment showed that litter carbon mass was the strongest controller on soil DOC content among litter quantity and quality indexes (Zhou et al. 2015).

Taken together, our results on temporal patterns of soil C and N content variations during root litter decomposition may reflect the balance between release from root litter and losses by microbes. The presence of root litter may accelerate the decomposition of soil organic matter, leading to the lack of soil C accumulation (Blagodatskaya and Kuzyakov 2008). Previous investigations on root decomposition mostly focused on root mass loss over time, with less attention to the effects of root litter decaying on microbial growth and activity (Manzoni et al. 2021). Comprehension of this synergistic effect may allow more robust predictions of how fine-root

litter decomposability affects soil C and N pool in future studies.

### Limitations of the study

The aim of the study was to assess the effects of root order on litter decomposition and further on soil C and N content. We recognized that there were certain limitations while exploring the objectives of the study. First, the use of buried litterbag technique may misrepresent the actual root decaying processes and the accompanying influences on soil. As shown by a recent comparative study, litterbags separated litter from soil may depress litter decomposition rate, especially of the finest roots (Chen et al. 2021), but this effect was inconsistent with other studies (Li et al. 2020). Therefore, novel research methods are needed for fine-root decomposition in situ. While litterbag method would likely be adopted in near future, it may be necessary to spread the roots without overlapping in the litterbags to maximize their contact with soil. Second, very small dry weight of root litter was used for incubation due to labor-intensive work required to collect sufficient amount of absorptive fine roots. The changes of soil nutrients might not be large enough for accurate detection by a small amount of input released from root decomposition. Therefore, the thin soil layer directly below litterbags would be appropriate sampling site to detect the impacts of C and nutrients release from root litter on soil. Third, as a laboratory incubation experiment focusing on discerning root-order effects on decomposition and on soil, the measured decaying rates might be underestimated for inter-comparisons (Chen et al. 2021). Long-term root decomposition of the distal roots in the field are preferred to understand the potential effects on SOM under the global changes.

### Conclusions

In summary, our data showed that the lower order roots of the climax dominant species *C. fargesii* decomposed significantly slower than the higher order roots, but this pattern was not true for the sub-climax dominant species *S. superba* roots. This finding highlighted that more work is needed on various species to reach a synthetic understanding of decomposition patterns across lateral root orders. For all the measured root litter traits, decomposition rates correlated best with C/N ratio in the studied species. In addition, root diameter and SRL were also closely related to root decay rate across root-order class. It should be emphasized that the underlying mechanisms of root decomposition may be not solely regulated by litter substrate quality, but also influenced by litter–soil contact. Soil C and N content obviously varied over the 540-day root decay period, whereas the effects of different-order-root decomposition on soil

C and N pool were not significant. Our research suggested that both litter-derived and microbial-derived C should be synergistically considered in evaluating the influence of root decomposition on soil C content. Consequently, further field studies are needed to investigate decomposition process of different-order roots and the consequent influence on soil C, N, and other nutrients with more species in diverse communities.

### Abbreviations

C: Carbon; N: Nitrogen; EF: Extractive fraction; ASF: Acid-soluble fraction; AIF: Acid-insoluble fraction; SEM: Structural equation modeling; EBLF: Evergreen broad-leaved forest; SOM: Soil organic matter; TNC: Total non-structural carbohydrate; DOC: Dissolved organic carbon; SRL: Specific root length; RO1–2: The first two-order roots; RO3: The third-order roots; RO4: The fourth-order roots; RO5: The fifth-order roots.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-022-00393-x>.

**Additional file 1: Table S1.** Correlations between initial root quality and root mass remaining (%) after 540 days of decomposition by linear regression analysis within each species and in the two species.

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### Author contributions

XC and YW conceptualized the idea and designed the study. YW led the writing of the manuscript. ZC, MZ and FW contributed to data collection and analyses. All authors contributed critically to the drafts and gave final approval for publication. All authors read and approved the final manuscript.

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### Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

All authors agreed and approved the manuscript for publication in *Ecological Processes*.

#### Competing interests

The authors declare that they have no competing interests.

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