## RESEARCH

# Influence of tree species on soil microbial residue accumulation and distribution among soil aggregates in subtropical plantations of China

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## Abstract

**Background** Microbial residues are significant contributors to stable soil organic carbon (SOC). Soil aggregates effectively protect microbial residues against decomposition; thus, microbial residue accumulation and distribution among soil aggregates determine long-term SOC stability. However, how tree species influence accumulation and distribution of soil microbial residues remains largely unknown, hindering the chances to develop policies for SOC management. Here, we investigated microbial residue accumulation and distribution in soil aggregates under four subtropical tree species (*Cunninghamia lanceolata*, *Pinus massoniana*, *Michelia macclurei*, and *Schima superba*) after 29 years of afforestation.

**Results** Accumulation of microbial residues in the 0–10 cm soil layer was 13.8–26.7% higher under *S. superba* than that under the other tree species. A structural equation model revealed that tree species affected the accumulation of microbial residues directly by altering fungal biomass. Additionally, tree species significantly affected microbial residue distribution and contribution to SOC in the top 20 cm soil. In particular, microbial residue distribution was 17.2–33.4% lower in large macro-aggregates (LMA) but 60.1–140.7% higher in micro-aggregates (MA) under *S. superba* than that under the other species in the 0–10 cm soil layer, and 14.3–19.0% lower in LMA but 43–52.1% higher in MA under *S. superba* than that under *C. lanceolata* and *M. macclurei* in the 10–20 cm soil layer. Moreover, the contribution of microbial residues to SOC was 44.4–47.5% higher under *S. superba* than under the other tree species. These findings suggest a higher stability of microbial residues under *S. superba* than that under the other studied tree species.

**Conclusions** Our results demonstrate that tree species influence long-term microbial persistence in forest soils by affecting accumulation and stabilization of microbial residues.

**Keywords** Tree species, PLFA, Amino sugar, Soil aggregate, Subtropical plantation

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## Introduction

Afforestation and reforestation are effective strategies for enhancing soil organic carbon (SOC) sequestration and mitigating climate change (Li et al. 2012; Steffens et al. 2021). Forest plantations have species-specific effects on litter characteristics and soil physicochemical properties (Sariyildiz et al. 2005; Wang et al. 2020a), leading to variable SOC sequestration under different tree species (Vesterdal et al. 2013; Pfeiffer et al. 2022). SOC pools comprise plant- and microbial-derived carbon. Conventionally, plants have been considered the major contributors to SOC stocks, with microbial-derived carbon having negligibly small contributions (Liang et al. 2017). However, the important contribution of microbial residues to the stable SOC pool is now recognized (Feng and Wang 2023). These residues are formed through an ex vivo modification pathway and stabilized through physical inclusion or chemical sorption (Liang et al. 2017). Their contribution to SOC pools can be approximately 60% in forest soils globally (Ni et al. 2020; Klink et al. 2022). Despite the recognized importance of microbial residues for long-term SOC sequestration (Jing et al. 2022; Klink et al. 2022), whether and/or how microbial residues are affected by tree species following long-term afforestation remains unclear. This research gap hinders the development of appropriate management policies to increase the strength of SOC sequestration and its potential for climate change mitigation.

To date, few studies have investigated the response of soil microbial residues across different forest types. A meta-analysis revealed significant differences in microbial residues between primary forests and plantations (Ma et al. 2022). However, direct evidence regarding the effects of tree species on microbial residue accumulation is limited, and the published data are controversial (Liang et al. 2006; Jing et al. 2018; Ma et al. 2022). Liang et al. (2006) and Jing et al. (2018) found that microbial residue accumulation is significantly influenced by tree species. Conversely, Yang et al. (2019) and Ma et al. (2022) showed that microbial residue accumulation did not differ between two plantations. These inconsistencies may be attributable to different climatic conditions and prior land use but are more likely attributable to differences in microbial communities and soil physicochemical properties, which are highly correlated with microbial residue accumulation (Wang et al. 2021; Ma et al. 2022). Therefore, it will be valuable to understand the mechanisms underlying microbial residue accumulation under different tree species. Moreover, current studies have primarily focused on bulk soils without considering the microbial residues distributed within soil aggregates. Soil aggregates, formed from minerals and organic compounds, effectively protect microbial residues from decomposition; however, the capability of soil aggregates for physical protection differs with aggregate size (Six et al. 2004; Ni et al. 2020). Hence, investigating the distribution of microbial residues among soil aggregates under various tree species will enhance current understanding of the SOC cycling process.

Soil aggregates are commonly classified into macro-aggregates ($> 0.25$ mm) and micro-aggregates ($< 0.25$ mm) (Six et al. 2004). Micro-aggregates are organo-mineral associations with lower turnover but higher stability and persistence, providing stronger physical protection than macro-aggregates (Six et al. 2004; Totsche et al. 2018). Microbial residue accumulation in micro-aggregates or distribution among aggregates are critical mechanisms for the long-term stabilization of microbial residues (Ni et al. 2020). Our current understanding of aggregate-associated microbial residues across different plantations is solely based on the study on temperate soils, which shows that introducing alder (*Alnus sibirica*) into larch (*Larix kaempferi*) plantations significantly increases microbial residues within all aggregate sizes (Jing et al. 2018). That study did not investigate whether microbial residue distribution differed between the two plantations, although tree species significantly affected the distribution of soil aggregates of different sizes (Jing et al. 2018; Su et al. 2021). Therefore, how microbial residue distribution responds to tree species remains an unaddressed question.

In subtropical China, *Cunninghamia lanceolata*, *Pinus massoniana*, *Michelia macclurei*, and *Schima superba* are the major afforestation species (Chen et al. 2014; Wang et al. 2020a). These species differ greatly in traits and characteristics, such as litter quality, soil microbial communities, and mineral elements (Wu et al. 2017; Zheng et al. 2018), and, in turn, these differences influence microbial residue accumulation, and SOC content. Although afforestation projects have been undertaken for decades, completely understanding the nature of the relationships among tree species, microbial residues, and SOC is elusive. In this study, we examined microbial residue accumulation and distribution in relation to microbial communities and soil properties in four core subtropical tree species to provide theoretical guidance for tree species selection. Our specific objectives were: (1) assessing species-specific effects on microbial residue accumulation and the factors controlling accumulation and (2) investigating species-specific effects on microbial residue distribution. We hypothesized that *S. superba* plantations have higher quantities of microbial residues than other plantation forests because of the higher lignin content in their litter (Wu et al. 2017; Zhu et al. 2021),

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which benefits microbial residue accumulation (Xu et al. 2022).

## Materials and methods

### Site description and soil sampling

The study was conducted at the Huitong Experimental Station of Forest Ecology, Hunan Province, Southern China (26°40′–27°09′N, 109°26′–110°08′E). The annual mean temperature in this region is 16.5 °C, with a maximum temperature of 27.5 °C and minimum temperature of 4.5 °C. The mean annual precipitation is 1200–1400 mm with most rainfall occurring between April and June (Wang et al. 2017). The soil in this region is classified as typical lateritic.

Until the 1920s, the climax vegetation of the region was evergreen broad-leaved forests dominated by *Cyclobalanopsis*, *Castanopsis*, *Lithocarpus*, and *Machilus*. Decades of excessive timber harvesting converted the natural forests to mixed broadleaf–conifer forests dominated by *Quercus fabri* and *Liquidambar formosana*. In the 1960s, the broadleaf–conifer forests were clear-cut and planted with *Cunninghamia lanceolata*. In 1989, four blocks with an area of 2 ha each were established in the *C. lanceolata* clear-cut site under the same initial conditions, and one of four species (i.e., *C. lanceolata*, *P. massoniana*, *M. macclurei*, and *S. superba*) was randomly allocated for planting in one of the four blocks. No treatment (e.g., fire or fertilization) was applied after afforestation.

In June 2018, four randomly selected plots (15 m × 15 m) were established within each tree species planted area, with distances between plots exceeding 100 m. The organic layer was removed, then eight soil cores were collected to 20 cm depth, divided into 0–10 and 10–20 cm depth segments, and homogenized to form a composite sample per plot for each depth. Subsequently, composite samples were passed through an 8-mm mesh sieve before soil aggregate fractionation. Subsamples were further passed through a 2-mm sieve and divided into two parts. One part was air-dried and ground for chemical and amino sugar analyses. The remainder was freeze-dried for the analysis of soil phospholipid fatty acid (PLFA).

### Separation of soil aggregates

Soil aggregates were separated using a field moist sieving method to maintain microbial activity during aggregate separation (Dorodnikov et al. 2009). Fresh soil (100 g) was placed in an aggregate sieving apparatus (Retsch AS200 Control, Retsch Technology, Dusseldorf, Germany) with 0.25- and 2-mm sieves stacked on top of each other, for 2 min. Then, soil on each sieve and below the 0.25 mm sieve were collected and weighed. The soil aggregates were divided into large macro-aggregates (LMA, > 2 mm), small macro-aggregates (SMA, 0.25–2 mm), and micro-aggregates (MA, < 0.25 mm).

### Measurements of soil characteristics

SOC and total nitrogen (TN) contents were measured with C/N analyzer (Elementar, Germany). Total phosphorus (TP) was quantified by ultraviolet spectrophotometer (UV-1700, Shimadzu, Japan) after $H_2SO_4$–$HCIO_4$ digestion. Available phosphorus (AP) and available potassium (AK) contents were measured by molybdenum blue colorimetry (Lu 2000) and ammonium acetate leaching-flame photometric method (Evangelou et al. 1994), respectively. Soil pH was measured using the soil:water suspension at a ratio of 1:2.5 (w/v). Soil exchangeable cations (EC, including $K^+$, $Na^+$, $Ca^{2+}$, and $Mg^{2+}$) were measured by the ammonium acetate method.

### PLFA analysis

Soil microbial communities were assessed via PLFA analysis according to Bardgett et al. (1996). Total PLFAs were extracted using a chloroform:methanol:phosphate buffer with a 1:2:0.8 ratio. After extraction, these PLFAs were analyzed using Agilent 6850GC (Agilent Technologies, Santa Clara, USA) and identified with MIDI software (MIDI Inc., Newark, DE, USA). PLFA markers were classified as saprotrophic fungi (SF, 18:2 w6c and 18:1 w9c), arbuscular mycorrhizal fungi (AMF, 16:1 w5c), actinomycetes (ACT, 16:0 10-methyl, 17:0 10-methyl, and 18:0 10-methyl) or other bacteria (a12:0, a13:0, i14:0, a14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0, 14:1w9c, 15:1w7c, 15:1w5c, 16:1w7c, 16:1w7c, 17:1w8c,17:0cy, 19:0cy,

**Table 1** The *p* values of two-way ANOVA for soil properties in plantations of different tree species

|  | SOC | C/N | C/P | N/P | AP | AK | EC | pH |
|---|---|---|---|---|---|---|---|---|
| Species | 0.936 | 0.759 | 0.864 | 0.937 | 0.011 | 0.775 | 0.077 | 0.542 |
| Depth | < 0.001 | < 0.001 | 0.002 | 0.188 | 0.004 | < 0.001 | 0.281 | 0.03 |
| Species × depth | 0.404 | 0.82 | 0.203 | 0.087 | 0.708 | 0.765 | 0.411 | 0.708 |

*SOC* soil organic carbon, *C/N* carbon:nitrogen ratio, *C/P* carbon:phosphorus ratio, *N/P* nitrogen:phosphorus ratio, *AP* available phosphorus, *AK* available potassium, *EC* exchangeable cations

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**Table 2** Soil properties at two depths in plantations of different tree species

| Species | Soil layer (cm) | SOC (g kg$^{-1}$) | C/N | C/P | N/P | AP (mg kg$^{-1}$) | AK (mg kg$^{-1}$) | EC (mg kg$^{-1}$) | pH |
|---|---|---|---|---|---|---|---|---|---|
| *C. lanceolata* | 0–10 | 25.11 ± 0.8 | 12.47 ± 0.46 | 41.45 ± 2.7 | 3.31 ± 0.1 | 4.96 ± 0.09 | 52.62 ± 3.32 | 346.95 ± 15.7 | 4.49 ± 0.03 |
| *P. massoniana* | | 26.51 ± 2.5 | 13.21 ± 0.82 | 37.46 ± 10.29 | 2.75 ± 0.64 | 5.62 ± 0.22 | 49.41 ± 3.56 | 288.63 ± 8.47 | 4.58 ± 0.11 |
| *M. macclurei* | | 23.33 ± 1.86 | 13.13 ± 0.45 | 36.53 ± 4.68 | 2.8 ± 0.41 | 4.93 ± 0.38 | 50.32 ± 1.79 | 305.9 ± 23.23 | 4.47 ± 0.02 |
| *S. superba* | | 26.58 ± 0.75 | 13.25 ± 0.15 | 48.8 ± 3.9 | 3.68 ± 0.3 | 3.80 ± 0.07 | 53.33 ± 2 | 263.2 ± 12.37 | 4.46 ± 0.02 |
| *C. lanceolata* | 10–20 | 15.59 ± 1.7 | 10.1 ± 0.33 | 25.22 ± 2.37 | 2.49 ± 0.15 | 4.20 ± 0.44 | 41.39 ± 6.97 | 295.9 ± 16.37 | 4.35 ± 0.05 |
| *P. massoniana* | | 12.8 ± 1.05 | 9.94 ± 0.37 | 33.19 ± 4.4 | 3.36 ± 0.51 | 4.57 ± 1.2 | 36.87 ± 2.2 | 314.46 ± 43.42 | 4.42 ± 0.11 |
| *M. macclurei* | | 14.05 ± 0.68 | 10.15 ± 0.21 | 29.04 ± 3.82 | 2.87 ± 0.44 | 3.05 ± 0.23 | 37.07 ± 6.63 | 271.24 ± 33.53 | 4.34 ± 0.03 |
| *S. superba* | | 12.69 ± 0.63 | 10.31 ± 0.63 | 24.18 ± 2.25 | 2.33 ± 0.1 | 2.71 ± 0.24 | 33.96 ± 2.61 | 249.66 ± 6.54 | 4.38 ± 0.01 |

Data were shown by mean ± SE

*SOC* soil organic carbon, *C/N* carbon:nitrogen ratio, *C/P* carbon:phosphorus ratio, *N/P* nitrogen:phosphorus ratio, *AP* available phosphorus, *AK* available potassium, *EC* exchangeable cations

12:0,14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0) (Olsson et al. 1995; Frostegård and Bååth 1996; Shao et al. 2019). Fungi were estimated as the sum of AMF and SF. Bacteria included ACT plus other bacteria. Total PLFAs were estimated as the sum of fungi and bacteria.

**Amino sugar analysis**

Amino sugars have been widely used to assess microbial residues (Jing et al. 2022; Liang et al. 2017). Amino sugars were determined according to Zhang and Amelung (1996). Soils containing 0.3 mg N were cultured with 6 M HCl for 8 h at 105 °C after which myoinositol was added as internal standard. The samples were then filtered and dried and the pH was adjusted to 6.6–6.8 with KOH; then, the samples were centrifuged at 3000 rpm for 10 min. The supernatant was dried again, dissolved in methanol, centrifuged at 3000 rpm for 10 min, and dried under N$_2$ gas. Amino sugars were derivatized as described by Jing et al. (2022). Subsequently, the amino sugar derivatives were analyzed using Agilent 7820A GC (Agilent Technologies, Santa Clara, CA, USA).

Muramic acid (MurA) and glucosamine (GluN) were used as bacterial and fungal residues index, respectively (Engelking et al. 2007; Ma et al. 2022). The molecular weights of GluN is 179.2. The bacterial residues, fungal residues and microbial residues were calculated as follows:

$$\text{Bacterial residues} \left(\text{mg g}^{-1}\right) = \text{MurN} \left(\text{mg g}^{-1}\right) \times 45, \tag{1}$$

$$\begin{aligned} \text{Fungal residues} \left(\text{mg g}^{-1}\right) = &(\text{mmol GluN} - 2 \\ &\times \text{mmol MurN}) \\ &\times 179.2 \times 9, \end{aligned} \tag{2}$$

$$\begin{aligned} \text{Microbial residues} \left(\text{mg g}^{-1}\right) \\ = \text{bacterial residues} \left(\text{mg g}^{-1}\right) \\ + \text{fungal residues} \left(\text{mg g}^{-1}\right). \end{aligned} \tag{3}$$

The distribution of microbial residues was calculated following Eq. 4 (Stemmer et al. 1998):

$$\text{CP}i(\%) = P_i \times \text{CC}_i \div \text{CC}, \tag{4}$$

where CP$i$ is the residues in the $i$th size fractions to the bulk soil, $P_i$ is the proportion of $i$ aggregates, CC$_i$ is microbial residue concentration in aggregates at the $i$th size (mg g$^{-1}$), and CC represents the residue concentration in bulk soil (mg g$^{-1}$).

**Statistical analysis**

A two-way analysis of variance (ANOVA) was performed to detect the effects of tree species, soil depth, and their interaction on soil properties, microbial community, and microbial residues. A three-way ANOVA was used to examine the effects of tree species, soil depth, aggregate sizes, and their interactions on microbial residue distribution among soil aggregates. Because their interaction was significant ($p < 0.05$), two-way ANOVA was further performed to analyze the distributions of microbial residues in each soil layer. Significant differences between 0–10 and 10–20 cm were identified using *t*-tests. Pearson's correlation analysis was conducted to explore the relationships between microbial residues and other measured parameters. Further, a structural equation model (SEM) was fitted to evaluate the direct and indirect effects of soil properties and microbial community on microbial residues. A SEM is acceptable when the $\chi^2$-test is non-significant and $\chi^2/df$ is between 0 and 2

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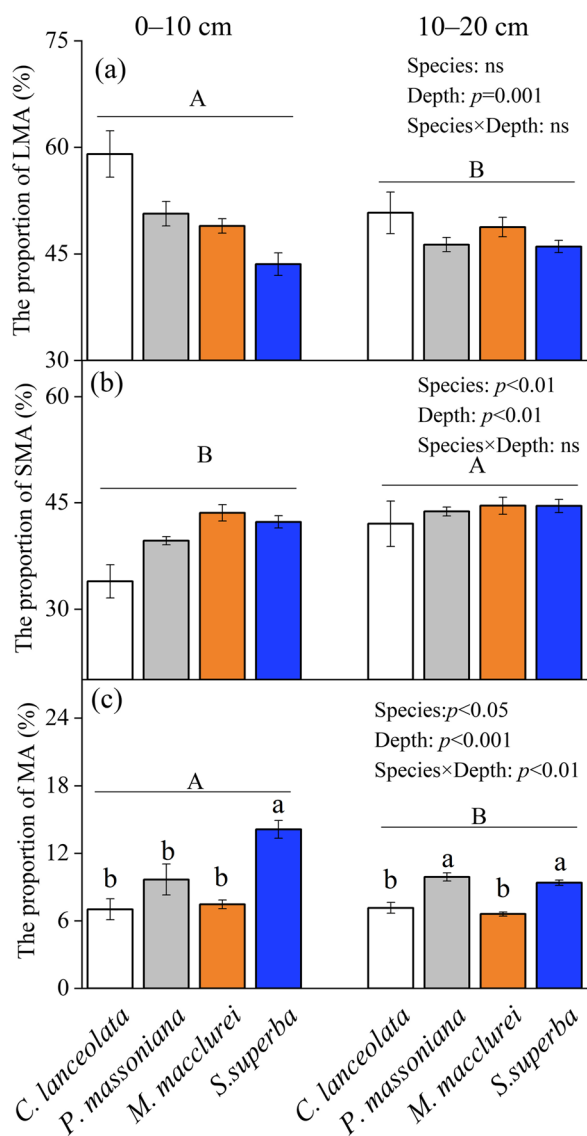


**Fig. 1** The proportion of LMA (**a**), SMA (**b**), and MA (**c**) in 0–20 cm soil layer of plantations of different tree species. Different lowercase letters indicate significant differences among tree species ($p < 0.05$). Values are mean ± SE. *LMA* large macro-aggregates, *SMA* small macro-aggregates, *MA* micro-aggregates

(Schermellehengel et al. 2003). The correlation and SEM analyses were conducted using the corrplot and lavaan packages, respectively, in R 3.4.1. Other statistical analyses were performed using SPSS 16.0 (SPSS Inc. Chicago IL, USA) software.

## Results

### Soil chemical properties and aggregate distribution

Apart from available phosphorus (AP), soil properties did not differ among tree species (Table 1). Specifically,

AP content was 23.2–39.8% lower under *S. superba* than that under *C. lanceolata*, *P. massoniana*, and *M. macclurei* plantations ($p < 0.05$; Table 2). Tree species did not affect the proportion of LMA but did affect the proportion of SMA ($p < 0.01$; Fig. 1b), with higher values in *M. macclurei* and *S. superba* than that in *C. lanceolata* plantations. Moreover, the interaction between tree species and soil depth affected the proportion of MA (Species×Depth: $p < 0.01$; Fig. 1c). The proportion of MA in the 0–10 cm soil layer was 14.1% in the *S. superba* plantation, which was significantly higher than that of 7.0–9.7% in the other plantations ($p < 0.01$; Fig. 1c). In the 10–20 cm soil layer, the MA proportion was 9.9% in the *P. massoniana* and 9.4% in the *S. superba* plantations, which were higher than the corresponding values of 7.2% and 6.6% in *C. lanceolata* and *M. macclurei* plantations, respectively ($p < 0.001$; Fig. 1c).

Soil depth significantly affected most soil chemical properties (Table 1). The SOC, carbon:nitrogen (C/N) ratio, carbon:phosphorus (C/P) ratio, AP, available potassium (AK), and pH were 89.7%, 28.5%, 41.7%, 32.8%, 37.8%, and 2.4% higher in the 0–10 cm than in the 10–20 cm soil layer ($p < 0.05$; Tables 1 and 2), respectively. Soil depth also significantly affected the distribution of soil aggregates ($p \leq 0.01$; Fig. 1). Specifically, the proportions of LMA and MA were greater, and the proportion of SMA was lower in the 0–10 cm layer than that in the 10–20 cm soil layer.

### Microbial community

Tree species neither affected total PLFAs nor bacterial biomass (Fig. 2a, b). However, tree species significantly affected the quantity of fungi and the fungi/bacteria ratio ($p < 0.05$ and $p < 0.001$, respectively; Fig. 2c, d). Specifically, the fungal biomass and the fungi/bacteria ratio were 0.86 µg g$^{-1}$ and 0.12, respectively, in the *S. superba* plantation, which were 16.1–41.4% and 18.4–33.5% higher than those in the other plantations, respectively. Moreover, the species effects depended on soil depth (Species×Depth: $p < 0.05$ and $p < 0.001$ for fungi and the fungi/bacteria ratio, respectively). For example, a larger fungal biomass (14%–68.3%) was observed in the 0–10 cm soil layer in the *S. superba* plantation than that in the other plantations but not in the 10–20 cm soil layer ($p < 0.05$; Fig. 2c).

Soil depth significantly affected the microbial groups and community structure. Across the different tree species sites, total PLFAs, fungal biomass, bacterial biomass, and fungi/bacteria ratios were 45.0%, 43.0%, 65.5%, and 14.5%, respectively, higher in the 0–10 cm soil layer than those in the 10–20 cm soil layer ($p < 0.05$; Fig. 2).

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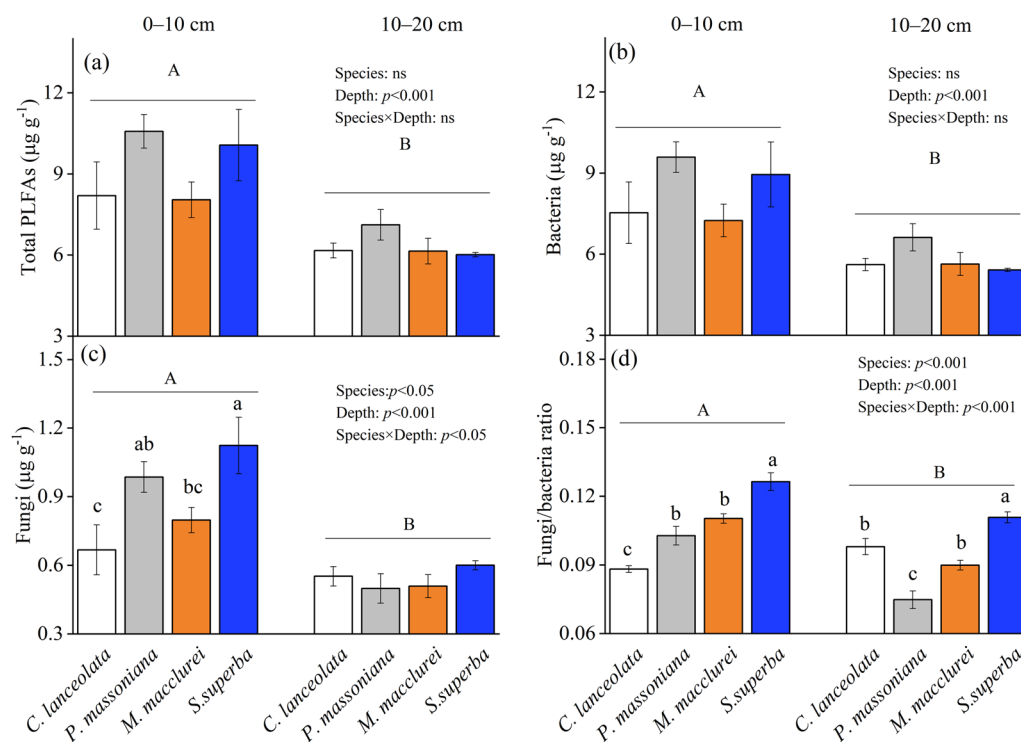


**Fig. 2** Biomass of total PLFAs (**a**), bacteria (**b**), fungi (**c**), and fungi/bacteria ratio (**d**) in 0–20 cm soil layer of plantations of different tree species. Different lowercase letters indicate significant differences among tree species ($p < 0.05$). Values are mean±SE

## Microbial residues and their distribution within soil aggregates

The accumulation and distribution of microbial residues were significantly associated with tree species. In the 0–10 cm soil layer, microbial residue accumulation was 13.8–26.7% higher under *S. superba* than that under the other species ($p < 0.05$; Fig. 3a). Moreover, microbial residue distribution in LMA was 17.2–33.4% lower ($p < 0.05$); however, that in MA was 60.1–140.7% higher ($p < 0.05$; Fig. 4) in the *S. superba* 0–10 cm soil layer than that in the other species. Additionally, residue distribution in LMA was 14.3–19.0% lower ($p = 0.05$); however, that in MA was 43–52.1% higher ($p < 0.05$; Fig. 4) in the 10–20 cm soil layer under *S. superba* than that under *C. lanceolata* or *M. macclurei*.

Tree species also affected the proportion of microbial residue in SOC. Microbial residues contributed to 53.0% of SOC in the *S. superba* plantation, which was higher than the contribution of 44.4–47.5% in the other species plantations ($p < 0.05$; Fig. 3b). In addition, soil depth significantly affected the proportion of microbial residue in SOC ($p < 0.05$; Fig. 3b). Although microbial residue accumulation in the 0–10 cm soil layer was 72.4% higher than that in the 10–20 cm layer ($p < 0.001$; Fig. 3a), the microbial residue contribution to SOC was 9.8% lower in

the 0–10 cm soil layer than that in the 10–20 cm layer ($p < 0.05$; Fig. 3b).

## Drivers of microbial residues in bulk soil

Microbial residues were closely associated with the soil properties and microbial community structure (Fig. 5). Specifically, microbial and fungal residues were both positively correlated with SOC, C/N ratio, AK, MA proportion, and specific microbial groups ($p < 0.05$; Fig. 5). Bacterial residues were positively correlated with these soil properties (except MA proportion) and microbial groups but negatively correlated with SMA proportion ($p < 0.05$; Fig. 5). The SEM showed that soil AK and fungi positively affected the fungal residues ($p < 0.01$; Fig. 6). Fungi also had a significant positive effect on the microbial residue accumulation, which further increased SOC content ($p < 0.01$; Fig. 6). Overall, the SEM explained 65%, 97%, and 89% of the variability in fungal residues, microbial residues, and SOC, respectively.

## Discussion

To date, the relationship between tree species and microbial residues in subtropical climate zones remains unclear, despite the urgent need to increase soil C sink strength by selecting appropriate tree species for
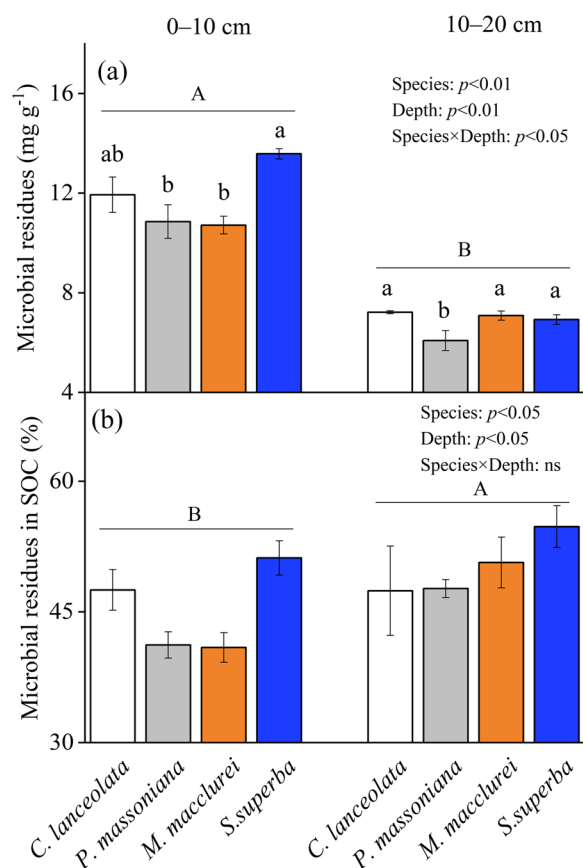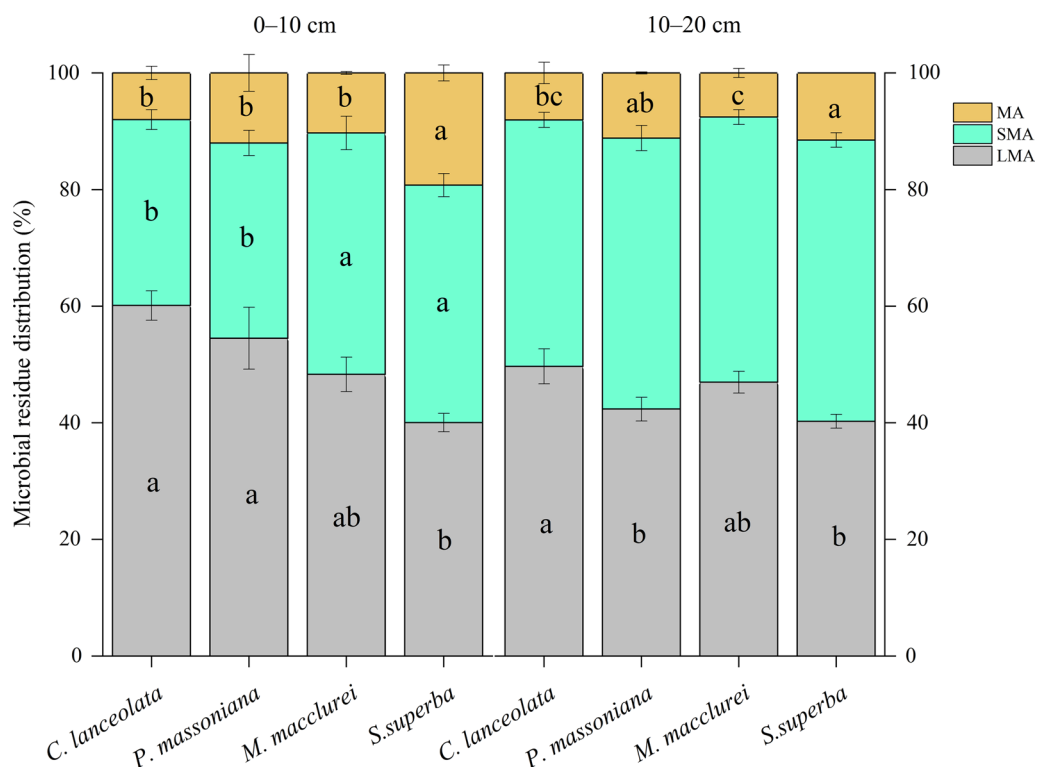
**Fig. 3** Microbial residues (**a**) and microbial residues in SOC (**b**) in bulk soil of different tree species. Different lowercase letters indicate significant differences ($p < 0.05$) among tree species. Values are mean ± SE

plantation management. This study provides a clear picture of the influence of tree species on accumulation and distribution of microbial residues in subtropical plantation forests. We found that the 0–10 cm soil layer under *S. superba* plantations was richer in soil microbial residues than were those under the other three plantation species (Fig. 3a), which supports our hypothesis and is consistent with previous studies on the influence of tree species on microbial residue accumulation (Liang et al. 2006; Jing et al. 2018). High microbial residues under *S. superba* can be attributed to the presence of increased fungal residues (Additional file 1: Fig. S1), which were positively correlated with fungal biomass (Fig. 6). Our result is in line with that of Ma et al. (2022), who reported that fungal biomass was positively related to microbial residues. Fungi can degrade recalcitrant litter, such as lignin (Strickland and Rousk 2010); thus, higher lignin content in the foliar litter of *S. superba* (24.8–36.4%) than in that of *P. massoniana*, *M. macclurei*, and *C. lanceolata* (Wu et al. 2017; Zhu et al. 2021) benefits fungal growth.

Second, the *S. superba* plantation has an extensive root system (Guo et al. 2022), which provides abundant labile C that can stimulate formation of fungal biomass (Bai et al. 2016; Jing et al. 2021). Increases in fungal biomass will consequently increase the production of fungal residues, as shown in our study. Furthermore, this process results in increased accumulation of microbial residues, as fungal residues contributed to most of the microbial residues (Additional file 1: Fig. S1, Ni et al. 2020). Another possible explanation for increased fungal residue accumulation being accompanied by a large pool of microbial residues is that fungi directly facilitate the accumulation of bacterial residues (Fig. 5). Fungi use their hyphae to explore and obtain limiting nutrients from other sources (Strickland and Rousk 2010), thereby reducing bacterial residue reutilization by living microbes, which usually occurs when microbes are constrained by nutrient availability (Wang et al. 2021). Moreover, dead fungal biomass can be directly utilized by bacteria (Zheng et al. 2021); thus, more fungi benefit bacterial residue formation. Notably, the SEM result provided the first direct evidence that fungi enhance SOC by increasing microbial residues, a relationship that was previously suspected (Strickland and Rousk 2010; Kallenbach et al. 2016). The positive correlation between the biomass of fungi and SOC content has an important ramification. Although changes in SOC under different tree species were not significant after 29 years of afforestation (Table 1), greater SOC sequestration under the *S. superba* plantation should be expected in future because of the relatively higher fungal biomass in the top 20 cm of the soil (Fig. 2). Therefore, continuous investigation of the effects of tree species on SOC in subtropical plantations is required.

In addition to high microbial residue accumulation in the floor under *S. superba*, we found that microbial residues contributed to 53% of SOC in this species plantation, which was the highest proportion among the four plantations (Fig. 3b). Given that microbial residues are relatively stable (Ni et al. 2020; Wang et al. 2021), our result suggests that the *S. superba* plantation has higher SOC stability than the other three. This conclusion is further supported by the distribution of microbial residues. The *S. superba* microbial residue in the top 20 cm soil was more widely distributed (43–140.7%) in MA (Fig. 5), which offers stronger physical protection than that in macro-aggregates (Six et al. 2004; Wang et al. 2020b). The higher distribution of microbial residues in MA in the 0–10 cm soil layer can be attributed to the higher MA proportion (Fig. 1) and higher microbial residue content under *S. superba* than that under the other species, whereas in the deeper soil layer, it can be explained only by the greater MA proportion (Additional file 1: Fig. S2). These findings suggest that soil aggregate distribution is

**Fig. 4** Distribution of microbial residues among aggregates in 0–20 cm soil layer of different tree species. Different lowercase letters indicate significant differences among tree species (*p* < 0.05). *LMA* large macro-aggregates, *SMA* small macro-aggregates, *MA* micro-aggregates

important for regulating microbial residue distribution and should be considered for the accurate prediction of soil C dynamics after long-term afforestation. Aggregate distribution depends on the combined effect of break-down and formation processes. In the current study, the higher MA proportion can be attributed to the greater breakdown of LMA in the *S. superba* than that in the other plantations, as evidenced by the opposing trends in the LMA and MA proportions (Fig. 1) and the significant but negative correlation between them (Fig. 5). Living roots, especially thin roots, are major drivers of aggregate formation and breakdown (Six et al. 2004; Wang et al. 2020b; Bai et al. 2021). They can promote macro-aggre-gate formation by bonding MA and particles together or by breaking macro-aggregates down and releasing MA during their growth through penetration (Leifheit et al. 2014; Kumar et al. 2017; Poirier et al. 2018). We found that *S. superba* had more fine roots (diameter < 1 mm) than the other species (unpublished data). Similarly, a previous study showed that *S. superba* has a consider-ably larger number of fine roots, especially thinner roots (diameter < 0.5 mm) than *C. lanceolata* (Yao et al. 2017). Although we did not estimate root-driven aggregate turn-over, a recent study showed that thinner roots increase the breakdown of macro-aggregates (Wang et al. 2020b).

Macro-aggregate breakdown releases large amounts of the highly bioavailable C fraction (Six et al. 2000), which can be adsorbed by MA (Guhra et al. 2019) and used by microbes within MA, thereby increasing microbial resi-dues in these aggregates. Adsorption should be stronger owing to the higher proportion of MA in the 0–10 cm layer than that in the 10–20 cm soil layer (Fig. 2). Conse-quently, microbial residues in MA increased in the upper soil layer.

We also found that AK had a positive effect on fun-gal residues (Fig. 6). Unlike soil depth, tree species did not affect soil AK (Table 1). Therefore, the increased accumulation of fungal residues in the upper layer than that in the 10–20 cm layer was due to the elevated AK content in the 0–10 cm layer. Potassium is the third most essential macronutrient, and an appropriate amount stimulates photosynthesis and facilitates root growth (Ma et al. 2013; Sattar et al. 2019). High AK content in the upper soil layer likely leads to increased root C input, and, consequently, to enhanced micro-bial metabolism. In this case, fungal residues, instead of bacterial residues, preferentially accumulate in soils because of the strong physical protection of aggregates (Jing et al. 2022), and recalcitrant substances in fungi hinder their residue decomposition (Fernandez et al.

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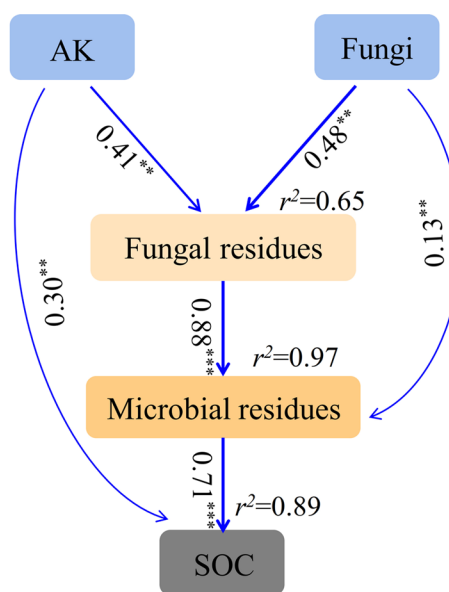
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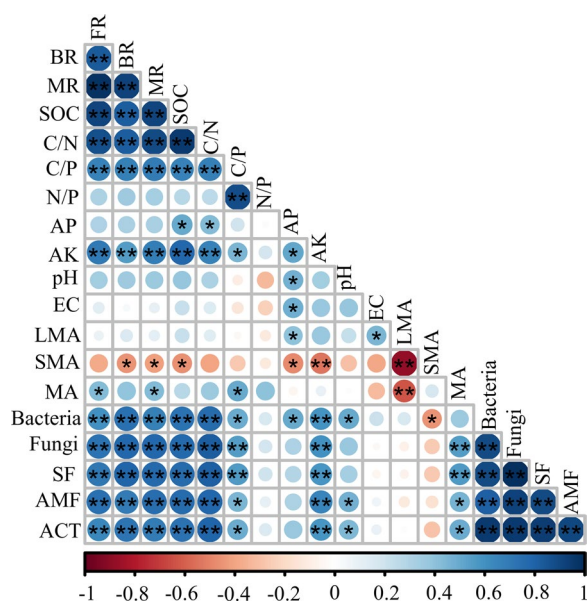


**Fig. 5** Pearson's correlation coefficient matrix of microbial residues and soil and microbial variables displayed with a color gradient. Significant levels are: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. *BR* bacterial residues, *FR* fungal residues, *MR* microbial residues, *SOC* soil organic carbon, *C/N* carbon:nitrogen ratio, *C/P* carbon:phosphorus ratio, *N/P* nitrogen:phosphorus ratio, *AP* available phosphorus, *AK* available potassium, *EC* exchangeable cations, *LMA* large macro-aggregates, *SMA* small macro-aggregates, *MA* micro-aggregates, *ACT* actinomycetes, *AMF* arbuscular mycorrhizal fungi, *SF* saprotrophic fungi



$p = 0.6$, CFI=1, RMSE=0

**Fig. 6** SEM for control of microbial residues and SOC in the top 20 cm soil of subtropical plantations. Red and blue arrows represent positive and negative relationships, respectively. The numbers next to arrows are standardized direct effects. Significant levels are: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. *ACT* actinomycetes, *EK* soil exchangeable K⁺, *CFI* comparative fit index

2019). Moreover, soil depth affected the proportion of microbial residues in SOC, which was 9.8% lower in the 0–10 cm layer than that in the 10–20 cm soil layer (Fig. 3b). This result highlights the significant contribution of microbial residues to SOC in deep layers, which aligns with the findings of recent studies (Ni et al. 2020; Wen et al. 2023).

## Conclusion

This study provides direct evidence of tree species-specific effects on microbial residue accumulation and stabilization in subtropical China. We found higher accumulation of microbial residue in *S. superba* plantations than that in the other plantations in the 0–10 cm soil layer, owing to the greater fungal biomass. Our results further showed that microbial residues were distributed extensively in micro-aggregates but less in large macro-aggregates, and the proportion of microbial residues in SOC in the top 20 cm soil was higher under *S. superba* than under the other species. These findings emphasize that *S. superba* plantations had higher microbial residue stability than other plantations. This study demonstrated the crucial role of tree species on long-term microbial residue sequestration.

This information has important implications for forest management practices to improve SOC in the subtropical region.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-023-00444-x.

**Additional file 1: Figure S1**. Fungal residues in bulk soil of plantations of different tree species. **Figure S2**. Microbial residues in micro-aggregates of plantations of different tree species.

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## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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