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Fine root litter quality regulates soil carbon storage efficiency in subtropical forest soils

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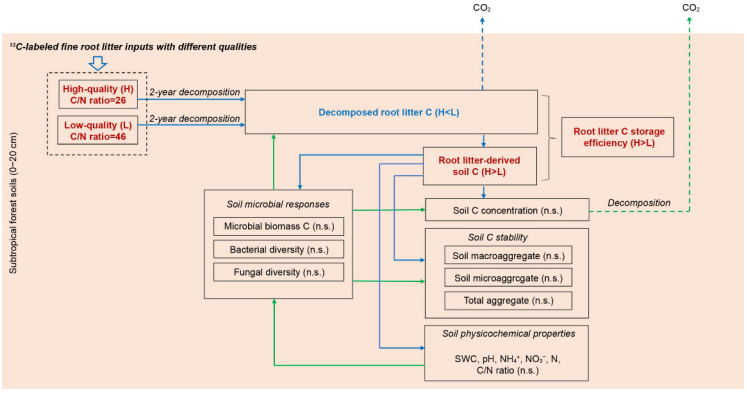
ABSTRACT

- High-quality and low-quality root litter had contrasting patterns of mass loss.
- Greater litter-derived C was incorporated into soils under high-quality root litter.
- Root litter decay rate or litter-derived C were related to soil microbial diversity.
- Root litter quality had little effect on soil physicochemical properties.
- High root litter quality was the main driver of enhanced soil C storage efficiency.



Decomposing root litter is a major contributor to soil carbon (C) storage in forest soils. During decomposition, the quality of root litter could play a critical role in soil C storage. However, it is unclear whether root litter quality influences soil C storage efficiency. We conducted a two-year greenhouse decomposition experiment using ^{13}C-labeled fine root litter of two tree species to investigate how root litter quality, represented by C to nitrogen (C/N) ratios, regulates decomposition and C storage efficiency in subtropical forest soils in China. 'High-quality' root litter (C/N ratio = 26) decayed faster during the first year (0−410 days), whereas 'low-quality' root litter (C/N ratio = 46) decomposed faster toward the end of the two-year period (598−767 days). However, over the two years of the study, mass loss from high-quality root litter (29.14 ± 1.42%) was lower than 'low-quality' root litter (33.01 ± 0.54%). Nonetheless, root litter C storage efficiency (i.e., the ratio of new root litter-derived soil C to total mineralized root litter C) was significantly greater for high-quality root litter, with twice as much litter-derived C stored in soils compared to low-quality root litter at the end of the experiment. Root litter quality likely influenced soil C storage via changes in microbial diversity, as the decomposition of high-quality litter declined with increasing bacterial diversity, whereas the amount of litter-derived soil C from low-quality litter increased with fungal diversity. Our results thus reveal that root litter quality mediates decomposition and C storage in subtropical forest soils in China and future work should consider the links between root litter quality and soil microbial diversity.

Keywords fine root litter quality, root litter decomposition, litter carbon storage efficiency, soil organic carbon accumulation, subtropical forest

1 Introduction

Fine root production represents a major component (40%−50%) of total net primary production in forests (Nadelhoffer and Raich, 1992; Majdi et al., 2005; McCormack et al., 2015). Fine root turnover and mortality lead to a large amount of fine root litter in the soils (Hendrick and Pregitzer, 1993; Burton et al., 2000), which accounts for 41% of annual litter inputs and represents an important input of carbon (C) to forest soil C pool (Schmidt et al., 2011; Freschet et al., 2012; Wang et al., 2018). Root litter has a high percentage of structural C compounds such as lignin, and therefore

often decomposes much more slowly than leaf litter (Hobbie et al., 2010; Bonanomi et al., 2021; Guo et al., 2021). The decomposition of root material can range from 2 to 11 years (Lin et al., 2010) because it represents a low-quality resource to microbial decomposers compared to leaf or needle litter (Bird et al., 2008; Xia et al., 2015; Bonanomi et al., 2021). Nonetheless, previous studies have demonstrated that root-derived C is retained more efficiently in soils and microorganisms than aboveground litter-derived C (Kramer et al., 2010; Mendez-Millan et al., 2010). As root litter decays belowground, it is generally better incorporated into soil aggregates, and more easily adsorbed to soil mineral surfaces than aboveground litter-derived C due to its close proximity with soil particles (Rasse et al., 2005; Sanaullah et al., 2011). Decaying root litter belowground could therefore be particularly important for soil C storage in tropical forests, where the conditions for decomposition are highly favorable and much of the C derived from aboveground litter is released to the atmosphere as CO_2 (Sayer et al., 2011; Xu et al., 2013; Liu et al., 2019). Therefore, understanding how root-derived C accumulates in soils during decomposition might provide valuable information about soil C storage in tropical forests.

There is increasing recognition that litter quality plays a critical role in litter decomposition rate and the subsequent storage of litter-derived C in the soil (Córdova et al., 2018; Yan et al., 2018; Zhou et al., 2019; Li et al., 2020; Craig et al., 2022) because the chemical and physical characteristics of the litter interact with the abiotic and biotic environment, i.e., soil physicochemical properties and microbial communities and diversity (van der Wal et al., 2013; Craig et al., 2022). For example, litter inputs of differing quality can alter soil pH and inorganic nitrogen (N) status depending on the types and levels of litter-derived acids, ions, dissolved organic C or inorganic N release (Carrillo et al., 2011; Tanikawa et al., 2018; Hensgens et al., 2021). In addition, litter quality can shape soil microbial community composition and diversity by providing distinct resources to microbial decomposers (Joly et al., 2016; Li et al., 2019). For example, Li et al. (2019) found that changes in litter stoichiometry with N availability increased the diversity of fungi but not bacteria, whereas other studies found that low-quality litter stimulates the growth of fungi over bacteria (Wardle et al., 2004; Poirier et al., 2018). Such interactions between litter quality and microbial communities are considered to be particularly important for soil C storage. For example, the emerging "soil-centered" approach posits that high-quality litter favors C accumulation in stable soil C pools due to high microbial C use efficiency (Cotrufo et al., 2013, 2019; Poirier et al., 2018). Previous studies have provided support for this theory, using isotopic tracers to compare the retention of root-derived and leaf-derived C in soils (Loya et al., 2004; Bird and Torn, 2006; Bird et al., 2008; Helfrich et al., 2008). However, other studies found that high-quality litter inputs favor soil C storage but the increase in soil C was not explained through microbial processes alone (Cotrufo et al., 2015; Craig et al., 2022). Nonetheless, more C from high-quality litter is incorporated into soil aggregates during the early stages of decomposition compared to low-quality litter (Gentile et al., 2011; Cotrufo et al., 2015). Although such studies are highly informative, they do not account for the longer decomposition times and often substantial differences in the quality of root litter among species, which could also influence soil C storage efficiency.

Whereas the importance of leaf litter quality has been extensively studied, we know much less about how variation in the quality of root litter among species controls decay rates and C storage efficiency, especially in the tropics (Sanaullah et al., 2011; Solly et al., 2014; Poirier et al., 2018; Guo et al., 2021). Differences in root litter quality among species and forest types (Hobbie et al., 2010; Sun et al., 2018; Cao et al., 2020) could explain some of the uncertainty around the incorporation of root-derived C into the soil C pool. At the same time, global environmental changes, such as warming, N deposition and precipitation change, are altering plant root chemistry (Bardgett et al., 2014; Wang et al., 2021) and plant community composition (Lu et al., 2010; Bertrand et al., 2011; Zhou et al., 2013, 2014; Franklin et al., 2016). In tropical regions, forest degradation and secondary regrowth after land clearance can result in markedly altered tree species composition (Martin et al., 2013). Such changes in plant community composition and diversity can alter fine root litter quality and thus can affect multiple soil properties, including soil organic C turnover and accumulation (Wallwork et al., 2022). Importantly, root litter quality determines the amount and type of compounds available to microbial decomposers and the distinct chemical composition of root litter from different species influences the accumulation of soil C (Bréchet et al., 2017). Differences in root litter quality are likely to play an important role in soil C storage because easily degradable compunds are accessible to a wide range of microorganisms, whereas more specialized fungal decomposers are required to degrade complex structural compounds (Hanson et al., 2008; van der Wal et al., 2013). Furthermore, as roots contain varying amounts of compounds that act as binding agents for soil aggregate formation (Rasse et al., 2005; Sanaullah et al., 2011), root litter quality could also influence the physical protection of new organic C inputs by inclusion in soil aggregates. Despite the potentially central role of root litter quality in soil C storage, most studies to date have focused on the influence of leaf litter quality (Elias et al., 2020; Huang et al., 2011; Liu et al., 2017; Tan et al., 2020a, 2020b). Given the importance of root litter as a key source of soil organic C in tropical forests (Silver et al., 2000; Bréchet et al., 2017), we urgently need to investigate how

fine root quality affects decomposition processes, soil properties and soil C storage efficiency in tropical forests.

Here, we conducted a two-year greenhouse experiment to examine how fine root litter quality, represented by the litter C/N ratio, regulates decomposition and C storage efficiency in subtropical forest soils. To compare decay rates and soil C storage efficiency, we used ^{13}C-labeled fine root litter of two subtropical tree species with distinct C/N ratios, which allowed us to track the incorporation of root litter-derived C into the soil. Specifically, we hypothesized that:

(H1) As high-quality root litter can be more easily decomposed by a wide range of soil microbes than low-quality litter, inputs of high-quality root litter will increase both microbial diversity and soil C storage efficiency;

(H2) Given that high-quality root litter contains more easily-decomposed C compounds that can act as soil binding agents, high-quality root litter inputs will result in greater formation of macroaggregates compared to low-quality root litter.

To support interpretation of our results, we also measured soil chemical properties to account for changes as a result of differences in soil C inputs and aggregate formation during the decomposition of high- and low-quality root litter.

2 Materials and methods

2.1 ^{13}C-labeled fine root litter of two tree species

We obtained fine root litter from two-year old saplings of two dominant tree species, *Cryptocarya chinensis* (Hance) Hemsl. and *Syzygium acuminatissimum* (Blume) DC., in a Monsoon evergreen broadleaved forest (23°10′N, 112°10′E) in subtropical China. We considered all live roots with a diameter < 2 mm as fine roots (McCormack et al., 2015). The C/N ratios of the root litter were 26.24 ± 0.30 for *C. chinensis* and 46.24 ± 0.40 for *S. acuminatissimum*, we henceforth refer to *C. chinensis* as high-quality root litter and to *S. acuminatissimum* as low-quality root litter. To label the root litter for the experiment, we placed the saplings in a closed greenhouse in South China Botanic Garden (23°8′N, 113°19.25′E) and continuously-labeled them with 10 atom‰ $^{13}CO_2$ for *c.* two months. Detailed methodology for the labeling is described in Soong et al. (2014). The fine roots of the tree saplings were harvested, oven-dried at 55°C for 48 h, ground to fine powder using a ball mill (Retsch MM400, Haan, Germany), and the $\delta^{13}C$ value was analyzed using EA-IRMS analysis (Elementar Vario Micro Cube-Isoprime 100 system) at the Scientific Instruments Sharing Platform, Third Institute of Oceanography, Ministry of Natural Resources, China. The $\delta^{13}C$ of *C. chinensis* and *S. acuminatissimum* root litter was −7.68 ± 0.06 ‰, and −0.37 ± 0.04‰, respectively (Table 1), which were both significantly higher than that of unlabelled mixed fine root litter at the study site (−29.81 ± 0.29‰) and of soil C at 0−20 cm depth (*c.* −27.55‰; Xiong et al., 2020), allowing us to quantify the C derived from fine root litter in the soil.

2.2 Experimental design

We collected surface soils (0−20 cm depth) from the same subtropical forest site. The soil was sieved (1-mm diameter sieve mesh) and homogenously mixed, then packed into PVC columns (22 cm height, 10 cm diameter, Fig. S1a), to 2 cm below the top rim; the bottom of each column was covered with a piece of 50-μm nylon mesh. To measure decomposition rates, the fine root litter from the labeled-tree saplings and cut into 4−5 cm lengths, and then placed in litterbags (7 cm × 7 cm; 50 μm mesh). To ensure we were able to trace the labeled C from the root litter, each litterbag contained 4.2 g fine root litter, which was twice the fine root production at the study site (i.e., 135 g m^{-2} per year, Deng et al., 2012, 2018). We prepared 24 litterbags (four replicates for each of six sampling times) per species and buried one bag per column at a depth of 5 cm. Thus, there were 48 columns with litterbags in total, comprising 24 replicate columns of two litter treatments (high-quality, low-quality). In addition, there were 24 control columns with soils but without litterbags. The decomposition experiment commenced on 26 March 2019 and lasted for two years. Air temperature (°C) and air relative humidity (%) in the greenhouse were automatically monitored every 15 min from January 2019 to July 2020 by HOBO data loggers (MX2301, HOBO, Onset, USA; Fig. S1b,c). We watered the soils with 85 mL purified water every two weeks based on the mean annual precipitation (1956 mm/yr) at the study site, such that soil water content ranged from 14% to 28% (Fig. 6a, see below). We destructively sampled four replicate column per treatment at each of six time points: 40, 98, 197, 410, 598 and 767 days since the start of the experiment.

Table 1 The initial carbon (C) content, nitrogen (N) content, C/N ratio and $\delta^{13}C$, of fine root litter from two tree species, *Cryptocarya chinensis* (high-quality root litter) and *Syzygium acuminatissimum* (low-quality root litter), from a subtropical forest in China.

Species	$\delta^{13}C$ (‰)	C (%)	N (%)	C/N
Cryptocarya chinensis	−7.68 ± 0.06	44.61 ± 0.10	1.70 ± 0.02	26.24 ± 0.30
Syzygium acuminatissimum	−0.37 ± 0.04	41.62 ± 0.07	0.90 ± 0.01	46.24 ± 0.40

2.3 Root litter properties

To determine root litter mass loss during decomposition, four replicate litterbags per treatment were collected on each sampling date, cleaned with distilled water, oven-dried at 55°C for 48 h, and weighed. The root litter decay rate k (year^{-1}) was calculated by the following equation:

$$k = \ln(L_0/L_t)/t, \quad (1)$$

where L_0 is the root litter mass at the beginning of the experiment (4.2 g dry weight), L_t is the remaining mass of root litter at each sampling time, and t is the number of days since the start of the experiment.

The root litter was then ground to fine powder using a ball mill (Retsch MM400, Haan, Germany), and total C and N were determined on a C/N analyzer (IsoPrime100, Elementar Analysen Systeme, Germany). The $\delta^{13}C$ value was analyzed using EA-IRMS analyses (Elementar Vario Micro Cube-Isoprime 100 system at the Scientific Instruments Sharing Platform, Third Institute of Oceanography, Ministry of Natural Resources).

2.4 Measurements of soil physical, chemical and microbial properties

To determine how root litter quality influences soil properties and microbial communities, we sampled the 5-cm section of soil immediately beneath the litterbag in each column. Due to restrictions during the Covid-19 pandemic, not all measurements could be made at the same time points. Soil pH was measured at each time point by a glass electrode pH meter (FE28, Mettler Toledo, Shanghai, China) in a 1:2.5 soil/water suspension. Soil inorganic N was determined at two sampling points (after 410 and 767 days of decomposition); soil inorganic N was extracted with 2 M KCl in a 1:10 soil:solution ratio and the filtrates were analyzed for NH_4^+-N and NO_3^--N by colorimetry (Lachat flow-injection autoanalyzer, Lachat Instruments, Mequon, WI, USA). To determine soil total C and N concentrations and C isotope ratios ($\delta^{13}C$), oven-dried samples were ground to fine powder using a ball mill (Retsch MM400, Haan, Germany), and the $\delta^{13}C$ value was analyzed by EA-IRMS (Elementar Vario Micro Cube-Isoprime 100 system at the Scientific Instruments Sharing Platform, Third Institute of Oceanography, Ministry of Natural Resources). Microbial biomass carbon (MBC) was determined at the first and last sampling time (after 40 and 767 days of decomposition) on a 15-g subsample of fresh soil from each column using the chloroform fumigation-extraction method (Vance et al., 1987). Soil C was extracted from paired 7.5-g subsamples of fumigated and unfumigated soils in 75 mL 0.5 M K_2SO_4 solution and the C in the extracts was determined using a TOC analyzer (High TOC, Elementar Analysen Systeme, Hanau, Germany). Microbial biomass C was calculated from the difference in extractable C concentrations between the fumigated and the unfumigated samples, using a conversion factor of 0.45 (Jenkinson, 1988).

Microbial diversity was determined at the final two sampling times (after 598 and 767 days of decomposition) following DNA extraction, PCR amplification, and amplicon sequencing at Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) according to standard protocols detailed in Hu et al. (2021). Briefly, DNA was extracted from 0.5 g samples with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, USA) following the manufacturer's instructions. For amplification, primers 338F and 806R were used for bacteria, and ITS1F and ITS2 for fungi. Amplification reactions were carried out in 4 μL of 5 FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer, 10 ng of template DNA, and 0.4 μL of FastPfu DNA Polymerase (TransGen Biotech., China; total volume 20 μL). Amplification was carried out on an ABI GeneAmp 9700 Thermal Cycler (Thermo Fisher Scientific, USA). PCR conditions were: 3 min at 95°C followed by 30 amplification cycles for bacteria (30 s denaturation at 95°C, 30 s annealing at 55°C, and 45 s at 72°C) or 35 amplification cycles for fungi (1 min denaturation at 94°C, 1 min annealing at 51°C, and 1 min at 72°C), and a final extension for 10 min at 72°C. PCR products were gel-purified (AxyPrep DNA Gel Extraction Kit; Axygen Biosciences, USA) and paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform (Illumina, USA). Sequences were quality-trimmed (average quality score > 20 over a 10 bp moving-window and a 50 base-pair minimum length) and processed to eliminate chimera following the procedure described by Hu et al. (2021). Sequences were binned into operational taxonomic units (OTUs) based on 97% pairwise identity using the UCLUST algorithm in the USEARCH package (Edgar, 2010). We used the number of OTUs in each sample to calculate the Shannon index as a measure of bacterial or fungal diversity (Hill et al., 2003).

2.5 Soil aggregate partitioning

We did soil aggregate partitioning on four sampling dates (after 40, 98, 197, and 767 days). We separated water-stable aggregates (WSA) into four size classes (> 2000 μm, 250–2000 μm, 53–250 μm and < 53 μm-diameter) using a wet-sieving apparatus (XY-100, Xiang yu Wei ye Instrument Equipment Co., Ltd., Beijing, China) with nested sieves of corresponding mesh sizes (Six et al., 1998, 2000). A 50-g air-dried soil sample was placed on the top sieve of each nest, submerged in water for 10 min and then the apparatus was shaken vertically (4 cm) 30 times per minute for 10 min. The soil retained in the three largest sieves was transferred to an aluminum tube, oven-dried at 60°C, and then weighed.

To separate the < 53-μm soil fraction from the distilled water, the buckets were left undisturbed for 8 h, allowing the < 53-μm soil fraction to settle at the bottom of the buckets; and after carefully pouring out the water, the sediment was transferred to aluminum cups, oven-dried and weighed. The detailed procedures are described in Xu et al. (2018). We defined macroaggregates as the sum of the 250–2000 and > 2000-μm aggregate fractions, and microaggregates as the sum of the < 53- and 53–250 μm aggregate fractions, and expressed them as mass percentages.

2.6 Root litter-derived C in soils, net changes in soil C and mineralized native soil C

Here, we defined root litter-derived C in soils as the C derived from fine root litter during the experiment. The amount of root litter-derived C in each column was calculated from the distinct $\delta^{13}C$ values (‰) of fine root C inputs and soil C using the following equation to partition the different C sources (Cheng, 1996):

$$Cn = Ct(\delta t - \delta s)/(\delta p - \delta s);$$

where *C*n is the amount of new soil C (g kg^{-1}) derived from fine root inputs, *C*t is the total soil C pool (g kg^{-1}) at the end of the experiment, *δ*t is the $\delta^{13}C$ value (‰) of the total soil C pool (*C*t) at the end of the experiment, *δ*s is the $\delta^{13}C$ value (‰) of the initial soil, and *δ*p is the $\delta^{13}C$ value (‰) of high- or low-quality root litter. Total mineralized root litter C (g) was calculated by the difference between initial root litter C content (initial litter mass (g) × initial litter C concentration (%)) and root litter C content at each sampling time (litter mass remaining (g) × litter C concentration (%)). Root litter C storage efficiency was calculated as the ratio of root litter-derived C incorporated into the soils to total mineralized root litter C (Stewart et al., 2007). Hence, high root litter C storage efficiency indicates that more root litter-derived C is stored in the soil instead of being released as CO_2. The net change in the soil C pool was calculated for the two root litter types from the difference in soil C concentrations at the beginning and the end of the experiment. The amount of mineralized extant soil C was calculated as the difference between new root litter-derived C stored in the soil and the net change in soil C. All figures show the means for four replicates per litter type and time point.

2.7 Statistical analysis

The data were checked for normality and homogeneity of variance and then we used two-way analysis of variance (ANOVA) to test the effect of sampling time, litter C/N ratio and their interactions on each litter response variable (litter decay rates and mass loss, litter C and N). We used the Least Significant Difference (LSD) tests to determine differences between control and litter types at each individual time point.

Finally, we used correlation analysis to determine relationships between soil properties and litter decomposition and the relationships between microbial diversity and litter decomposition rate or litter-derived C. All statistical analyses were conducted in R version 3.5.1 (R Development Core Team 2018) and all the figures were created in Sigmaplot 12.5 (Systat Software Inc., San Jose, California, USA).

3 Results

3.1 Changes in root litter C and N concentrations and root litter decay rates

The C and N concentrations of high-quality (*C. chinensis*) root litter were significantly higher and the C/N ratio was consistently lower than those of low-quality (*S. acuminatissimum*) root litter throughout the experiment (Table 2, Fig. 1a, b, c). For both root litter types, the C and N concentrations in the remaining mass increased over time (Fig. 1a, b), whereas the C/N ratio declined (Fig. 1c).

At the end of the experiment (after 767 days), mass loss of the high-quality root litter was 29.14 ± 1.42% whereas mass loss of the low-quality root litter was 33.01 ± 0.54% (Fig. 1d). There was a significant interaction between root litter quality and sampling time for both mass loss (Table 2, Fig. 1d, P = 0.0017) and decay rate (k-values; Table 2, Fig. 1e, P = 0.0055) indicating differences in the temporal pattern of decomposition depending on root litter quality. Mass loss during the first 200 days was much faster for the high-quality than the low-quality root litter, but there was a late peak in the mass loss of low-quality root litter after the 410-day sampling point (Fig. 1). Accordingly, the decay rate of high-quality root litter was greater than that of the low-quality root litter during the first half of the experiment (before 410 days).

Table 2 The results for two-way analysis of variance (ANOVA) for litter variables, including litter carbon (C) concentration, litter nitrogen (N) concentration, litter carbon to nitrogen ratio (C/N), litter mass loss, litter decay rate (k), decomposed litter carbon (C). Treatment and time effects were considered significant at $P < 0.05$ (shown in bold type).

	Litter C (%)	Litter N (%)	Litter C/N	Litter mass loss (%)	Decay rate k	Decomposed litter C (%)
Sampling time	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
Litter quality	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.60022	**0.02399**	0.23044
Time × Litter quality	0.629	0.902	**< 0.0001**	**0.00166**	**0.00546**	**0.00322**

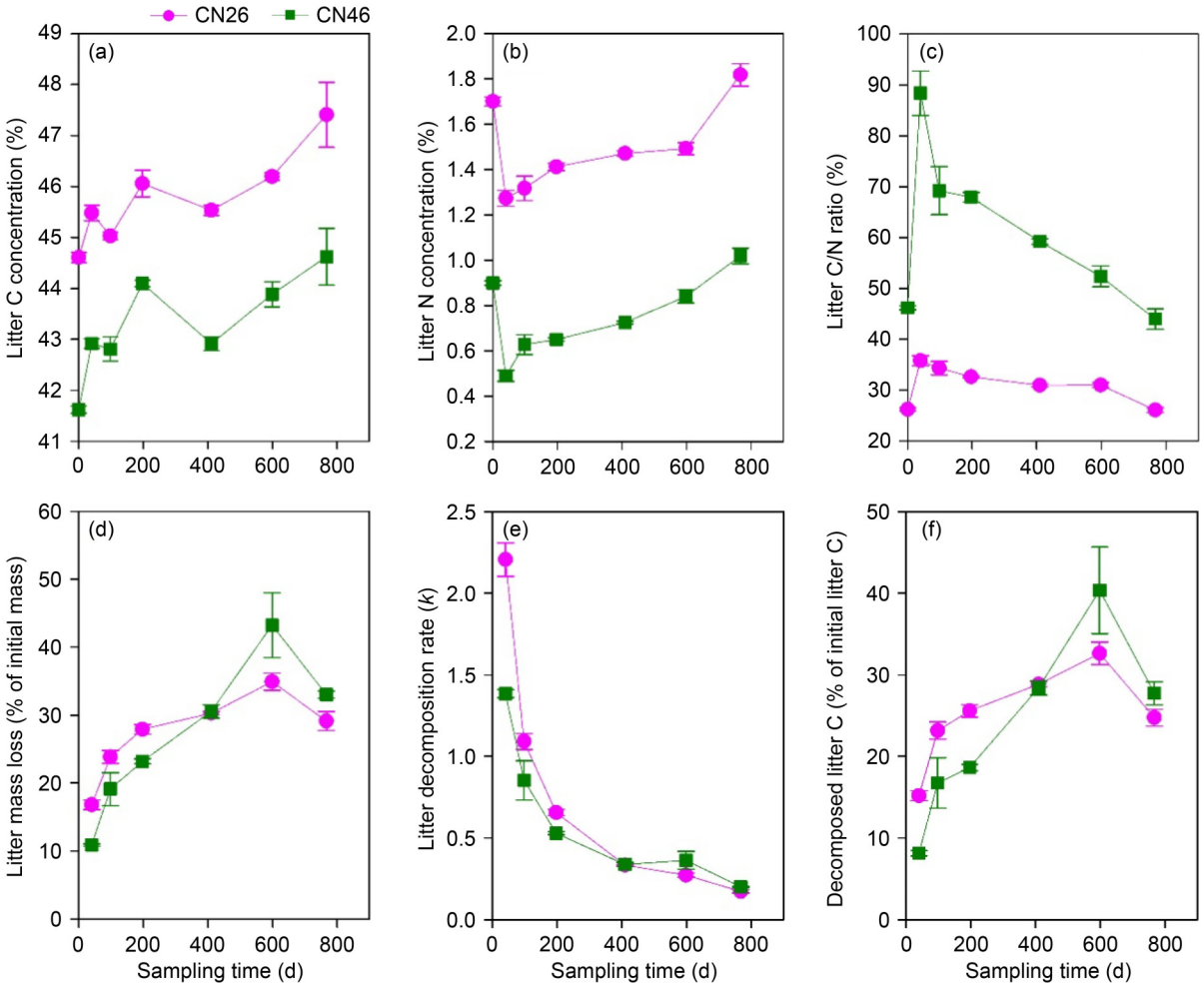


Fig. 1 Changes in root litter properties during two years of decomposition, showing (a) carbon (C) concentration, (b) nitrogen (N) concentration, (c) C/N ratio, (d) mass remaining, (e) decay rate (*k*), and (f) decomposed litter C at six time points (40, 98, 197, 410, 598 and 767 days since the start of the experiment). CN26 indicates high-quality litter with a C/N ratio of 26.24; and CN46 indicates low-quality litter with a C/N ratio of 46.24. Symbols and error bars represent means and standard errors for $n = 4$ per sampling point and root litter type.

However, after 410 days, the two root litter types had similar decay rates (Table 2, Fig. 1e). Root litter C loss during decomposition largely mirrored root litter mass loss (Fig. 1f).

3.2 Changes in soil carbon concentration and turnover

Neither high- nor low-quality fine root litter addition increased total soil C concentrations during the experiment (Table S1, Fig. 2a) and soil C concentrations did not differ between the two root litter types at any sampling time (Table S1). Surprisingly, the final soil C concentrations for both root litter types (15.6 ± 0.39 g kg^{-1} for high-quality root litter and 15.0 ± 0.42 g kg^{-1} for low-quality root litter), were slightly lower than the initial levels (16.03 ± 0.43 g kg^{-1}; Fig. 2a). Net soil C change followed the same pattern as soil C concentration (Table S1; Fig. 2b), whereas mineralized native soil C showed the reverse pattern to soil C concentration (Fig. 2c).

Soil $\delta^{13}C$ values under both types of fine root litter were significantly higher than initial levels but there was no difference between litter types (Table S1, Fig. 2d). Root litter-derived C in soils increased during the first half of the experiment (from days 40 to 410), then declined (Fig. 2e). However, toward the end of the experiment, significantly more root litter-derived C was stored in soils under high-quality (0.40 ± 0.03 g kg^{-1}) than low-quality root litter (0.13 ± 0.02 g kg^{-1}; Table S1). Consequently, litter C storage efficiency at the end of the experiment was significantly greater for high-quality (0.70 ± 0.085) than for low-quality root litter (0.18 ± 0.022; $P < 0.001$, Fig. 2e).

3.3 Changes in soil microbial biomass carbon and diversity

Microbial biomass C declined markedly between the first and last sampling times (time effect: $P < 0.000\,1$, Fig. 3a), but was not affected by root litter addition or root litter quality

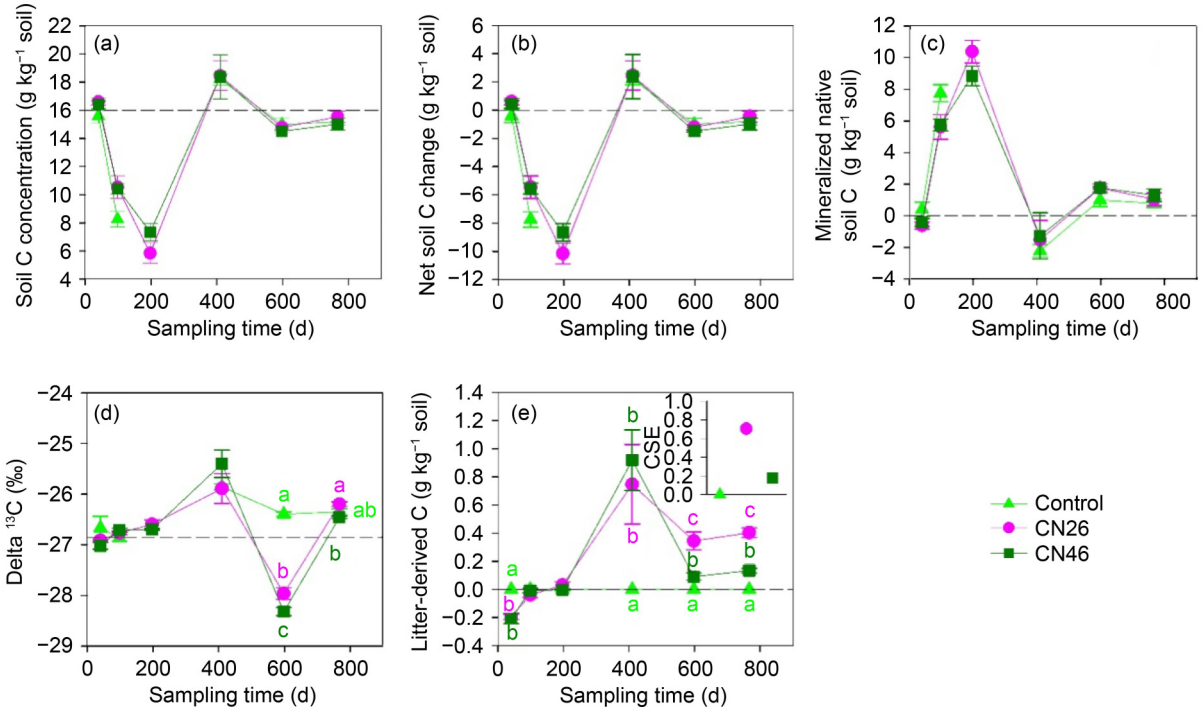


Fig. 2 Changes in soil carbon (C) during two years of root litter decomposition, showing (a) soil C concentration, (b) net soil C change, (c) mineralized native soil C, (d) soil $\delta^{13}C$, and e) root litter-derived soil C, at six time points (40, 98, 197, 410, 598 and 767 days since the start of the experiment). CN26 indicates high-quality litter with a C/N ratio of 26.24; and CN46 indicates low-quality litter with a C/N ratio of 46.24. Lower-case letters indicate significant differences between litter types and/or controls, where light green letters represent controls, purple letters represent CN26 and dark green letters represent CN46. Symbols and error bars represent means and standard errors for n = 4 per sampling point and root litter type.

(Table S1, Fig. 3a). Both bacterial and fungal diversity increased at the end of the experiment (time effect: $P = 0.000\,9$ and $P < 0.000\,1$, respectively), but there was no effect of root litter quality (Table S1, Fig. 3b, c). However, the decay rate of high-quality root litter declined with increasing diversity of bacteria ($R^2 = 0.53$, $P = 0.04$) and fungi ($R^2 = 0.65$, $P = 0.02$; Fig. 4a, b). There was no relationship between litter-derived C and bacterial diversity (4c) but the amount of soil C derived from low-quality root litter increased marginally with fungal diversity ($R^2 = 0.47$, $P = 0.06$; Fig. 4d).

3.4 Changes in soil macro-, micro- and total aggregates

Root litter addition did not increase total soil aggregates relative to the controls. Indeed, the mass percentage of aggregates declined under both root litter types during the experiment (time effect: $P < 0.000\,1$) and by the final sampling time (767 days), the mass percentages of soil macroaggregates and total aggregates under both types of root litter were lower than in the controls (Table S1, Fig. 5a, b, c). However, the mass percentage of aggregates under high-quality root litter (78.7 ± 0.87% and 79.6 ± 0.73%) was marginally higher than under low-quality root litter during the first 98 days (76.0 ± 0.53% and 76.5 ± 1.12%; Table S1, Fig. 5a). The mass percentage of macroaggregates differed with litter C/N ratio, sampling time and their interaction (Fig. 5b), whereby the mass percentage of macroaggregates at the first two sampling times was significantly higher under high-quality (45.4 ± 4.16% and 42.0 ± 2.51%) than low-quality root litter (35.3 ± 2.43% and 32.8 ± 2.42%). However, by the end of the experiment the mass percentage of macroaggregates was significantly lower under high-quality root litter (20.4 ± 0.60%) compared to low-quality root litter and 23.6 ± 0.79%; Table S1, Fig. 5b). The mass percentage of microaggregates was not affected by root litter quality (Fig. 5c).

3.5 Changes in soil water content and chemical properties

Soil water content was higher in soils with root litter relative to the controls throughout the experiment, but there was no effect of litter quality on soil water content (Table S1, Fig. 6a). Neither soil pH nor total soil N were affected by root litter addition or litter quality, but both changed markedly during the course of the experiment (Table S1, Fig. 6b, c). Soil nitrate-N concentrations (two time points only) did not differ between sampling points or root litter types (Fig. 6d) but soil ammonium-N concentrations were significantly higher under high-quality (0.27 ± 0.01 mg N L^{-1}) than low-quality root litter at the 410-day sampling point (Fig. 6e). The soil C/N ratio was also unaffected by root litter quality and followed the pattern for total soil N in the early stages of decomposition. However, the soil C/N ratio increased in the

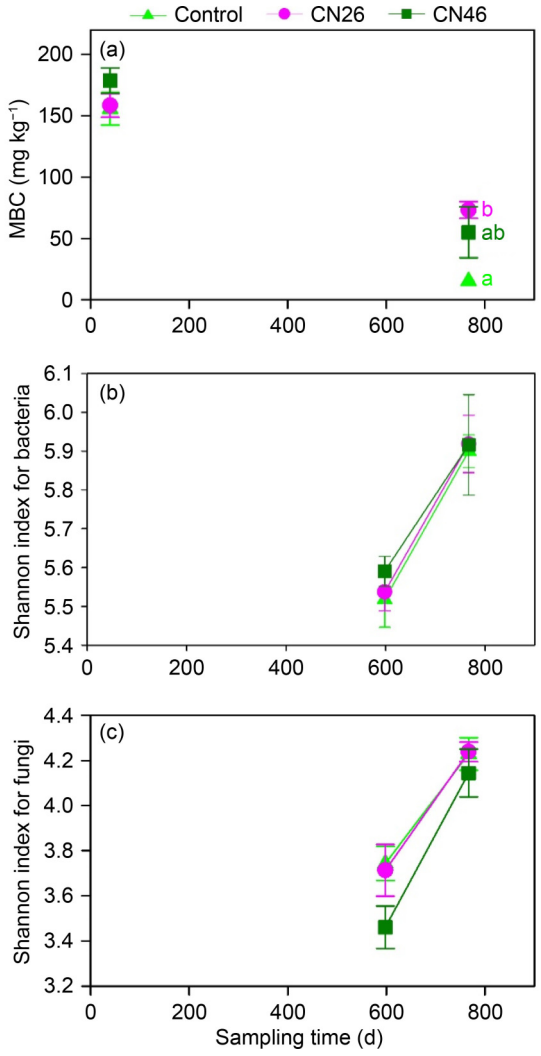


Fig. 3 Changes in soil microbial properties during two years of root litter decomposition, showing (a) microbial biomass carbon (MBC), and the diversity of (b) soil bacteria and (c) fungi at two time points (40 or 598 and 767 days since the start of the experiment), where CN26 indicates high-quality litter with a C/N ratio of 26.24; and CN46 indicates low-quality litter with a C/N ratio of 46.24. Symbols and error bars represent means and standard errors for n = 4 per sampling point and root litter type. Lower-case letters indicate significant differences between litter types, where purple letters represent CN26 and dark green letters represent CN46.

later stages of decomposition (from 410 to 767 days; P < 0.000 1, Fig. 6f), such that the soil C/N ratio at the end of the experiment was significantly higher than that in the first sampling time (Fig. 6f). Thus, root litter quality had no effect on soil chemical properties except ammonium-N concentrations.

4 Discussion

Our study demonstrates that differences in root litter quality, represented by C/N ratios, influence both litter decay rate and C storage efficiency in a subtropical forest soil. Our results show that high-quality root litter decomposes at a faster rate at the initial stages of decomposition but has a higher C storage efficiency at the later stages of decomposition compared to low-quality root litter. Although root litter quality had little influence on soil properties, high-quality litter was associated with macroaggregate formation during the early stages of decomposition and with higher concentrations of ammonium-N in the later stages. Overall, our findings demonstrate that high-quality root litter promotes the storage of new C in soils, but the differences in soil C storage efficiency could not be explained by macroaggregate formation, soil physicochemical properties or changes in soil microbial diversity. Thus, our results expand the prevailing view that high quality litter promotes soil C storage by demonstrating the influence of root C/N ratios on soil C storage efficiency and highlighting avenues for further investigation into the underlying mechanisms.

4.1 Fine root litter quality regulates decay rates and carbon storage efficiency in a subtropical forest soil

In support of our first hypothesis (H1), high-quality root litter decayed more rapidly than low-quality root litter and resulted in greater soil C storage efficiency by the end of the study. The rapid mass loss of high-quality litter during the initial stages of decomposition (0−197 days) can be attributed to the release of soluble and easily decomposable compounds (Bird and Torn, 2006; Bird et al., 2008). The reduction in decay rates and the lower mass loss from the high-quality litter compared to the low-quality litter after 410 days (Fig. 1d, e, f) indicates that most labile compounds had been mineralized leaving more lignified structural C (Lin et al., 2010). Hence, rapid mass loss of high-quality root litter during the early stages of decomposition does not necessarily entail faster overall decay (Luo et al., 2017), and short-term studies could therefore produce misleading decay rates for root litter.

The unusually high mass loss measured at 600 days is likely an experimental artifact. We measured mass loss by destructively sampling replicates at each time point, so the mass loss of individual samples was likely influenced by distinct conditions depending on their location in the greenhouse. Although we made every effort to distribute the replicates for each treatment equally throughout the greenhouse, differences in exposure to sunlight (and therefore temperature) likely account for the high mass loss of litter sampled at 600 days.

Despite the two-year experimental duration, both root litter types had lost less than half of their mass by the end of the study. Few other studies have conducted long-term (⩾ 2 years) root litter decomposition experiments in tropical forests but the mass loss for high-quality (29.14 ± 1.42%)

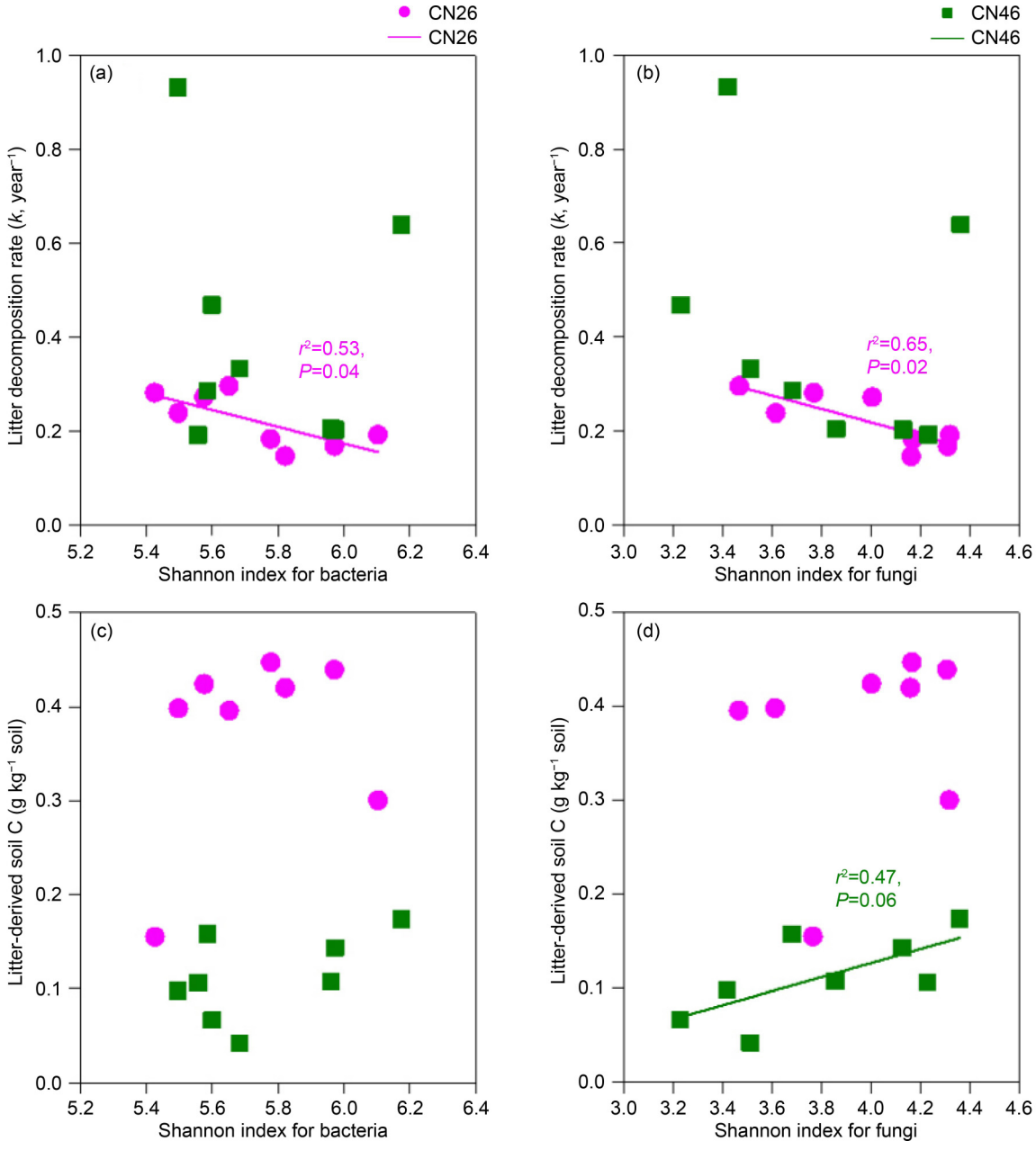


Fig. 4 Relationships between (a,b) litter decay rates or (c,d) litter-derived soil carbon (C) and the diversity of (a,c) bacteria or (b,d) fungi in the soil under different types of root litter. CN26 indicates high-quality litter with a C/N ratio of 26.24; and CN46 indicates low-quality litter with a C/N ratio of 46.24. Lines indicate significant relationships at $P < 0.05$. The purple line represents the relationship for CN26 and the green line represents the relationship for CN46.

and low-quality (33.01 ± 0.54%) root litter (Fig. 1d) under subtropical conditions was within the wide range reported for temperate forest sites in Switzerland (18−71%; Heim and Frey, 2004), and subtropical forest sites in China (20−40%; Wu et al., 2022). The mass loss of both root litter types fit the exponential model (Fig. 1e), and our experiment therefore spanned the decomposition stages that are considered to be regulated by litter nutrient concentrations (e.g., N), rather than litter lignin concentrations (Berg, 1984; Taylor et al., 1989). The marked decline in litter N concentrations in both litter types during the first 40 days of decomposition indicates initial N mineralisation (Chen et al., 2002) rather than immobilisation, indicating that N is unlikely to be limiting to litter decomposition at our site (Fang et al., 2009). Nonetheless, given that < 50% mass was lost from the root litter during the study, our two-year experiment still represents the early stages of fine root litter decomposition. As the amounts of recalcitrant C compounds in litter likely play an increasingly important role as decomposition progresses (Harmon et al., 2009), longer-term experiments are necessary to reveal the dynamics of root C and N content during the later stages of decay.

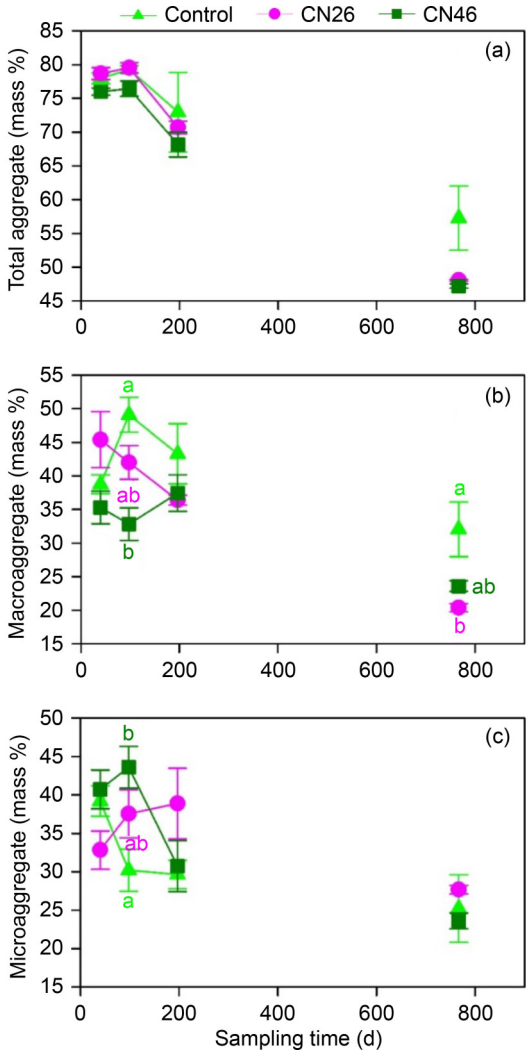


Fig. 5 Changes in the mass percentages of soil aggregates during two years of root litter decomposition, showing (a) total aggregates, (b) macroaggregates, and (c) microaggregates at four time points (40, 98, 197 and 767 days since the start of the experiment), where CN26 indicates high-quality litter with a C/N ratio of 26.24; and CN46 indicates low-quality litter with a C/N ratio of 46.24. Symbols and error bars represent means and standard errors for n = 4 per sampling point and root litter type. Lower-case letters indicate significant differences between litter types, where purple letters represent CN26 and dark green letters represent CN46.

In accordance with hypothesis H1, root litter quality clearly influenced the incorporation of litter-derived C into the soil, as greater amounts of litter-derived C, and thus higher C storage efficiency, were observed for the high-quality root litter by the end of our experiment (Fig. 2e). Despite this, root litter quality had little effect on total soil C concentration, native soil C mineralization, or net soil C change (Fig. 2a, b, c). The substantial mineralisation of native C in all soils during the first 200 days of the experiment (Fig. 2c) can be attributed to soil disturbance by sieving, which breaks up the soil structure, making more of the soil organic matter available to microbial attack (Thomson et al., 2010). The concommitant decline in total N provides further evidence for the initial mineralisation of extant soil organic matter. Nonetheless, the greater amount of soil C derived from high-quality litter by the end of the study supports the prevailing view that high quality litter inputs enhance soil C storage (Cotrufo et al., 2013, 2015, 2019; Craig et al., 2022).

Greater soil C storage under high-quality litter inputs is thought to result from greater microbial turnover of easily degradable compounds during decomposition Cotrufo et al., 2013, 2015, 2019; Craig et al., 2022). However, the evidence in support of our hypothesis that litter quality would be associated with microbial diversity (H1). We found that faster decay of the high-quality litter was associated with lower bacterial diversity (Fig. 4a) but that new soil C derived from low-quality root litter was associated with higher fungal diversity (Fig. 4d). This discrepancy could be because we were only able to assess microbial diversity at the end of the experiment, when the decay rates of the high-quality litter had already declined substantially (Fig. 1e). High decay rates at low microbial diversity could reflect the predominance of efficient litter decomposers (Hättenschwiler et al., 2005), as specialist decomposer organisms take a central role once easily degradable compounds have already been released from the litter (Herzog et al., 2019). The high proportion of fungi in soils under low quality litter exemplifies the importance of fungi as specialist decomposers, and corresponds to the lower nutrient requirements and higher C-use efficiency of fungi compared to bacteria (Keiblinger et al., 2010; Fanin et al., 2014). Thus, our findings indicate that low-quality litter can contribute to soil C stabilization by stimulating the growth of fungi over bacteria (Wardle et al., 2004; Poirier et al., 2018). It is possible that microbial diversity will become increasingly important in the late stages of root decomposition (> two years), as specialized microorganisms are required to degrade the remaining lignified C compounds (Voříšková and Baldrian, 2013; Fanin et al., 2016).

4.2 Fine root litter quality has limited influence on soil aggregate formation

We found little evidence to support our hypothesis that high-quality root litter will promote the formation of soil aggregates (H2). The larger macroaggregate fraction under high quality litter during the first 98 days mirrors the findings of a previous study using leaf litter, in which more C from high-quality litter was incorporated into soil aggregates during the first three months of decomposition (Gentile et al., 2011). The process of macroaggregation during litter decomposition is transient because disaggregation commences as easily-available C is consumed and microbial activity decreases (Helfrich et al., 2008). Accordingly, the percentage of macroaggregates and total aggregates in our experiment declined over time (Table S1, Fig. 5a, b). It is also noteworthy that the larger macroag-

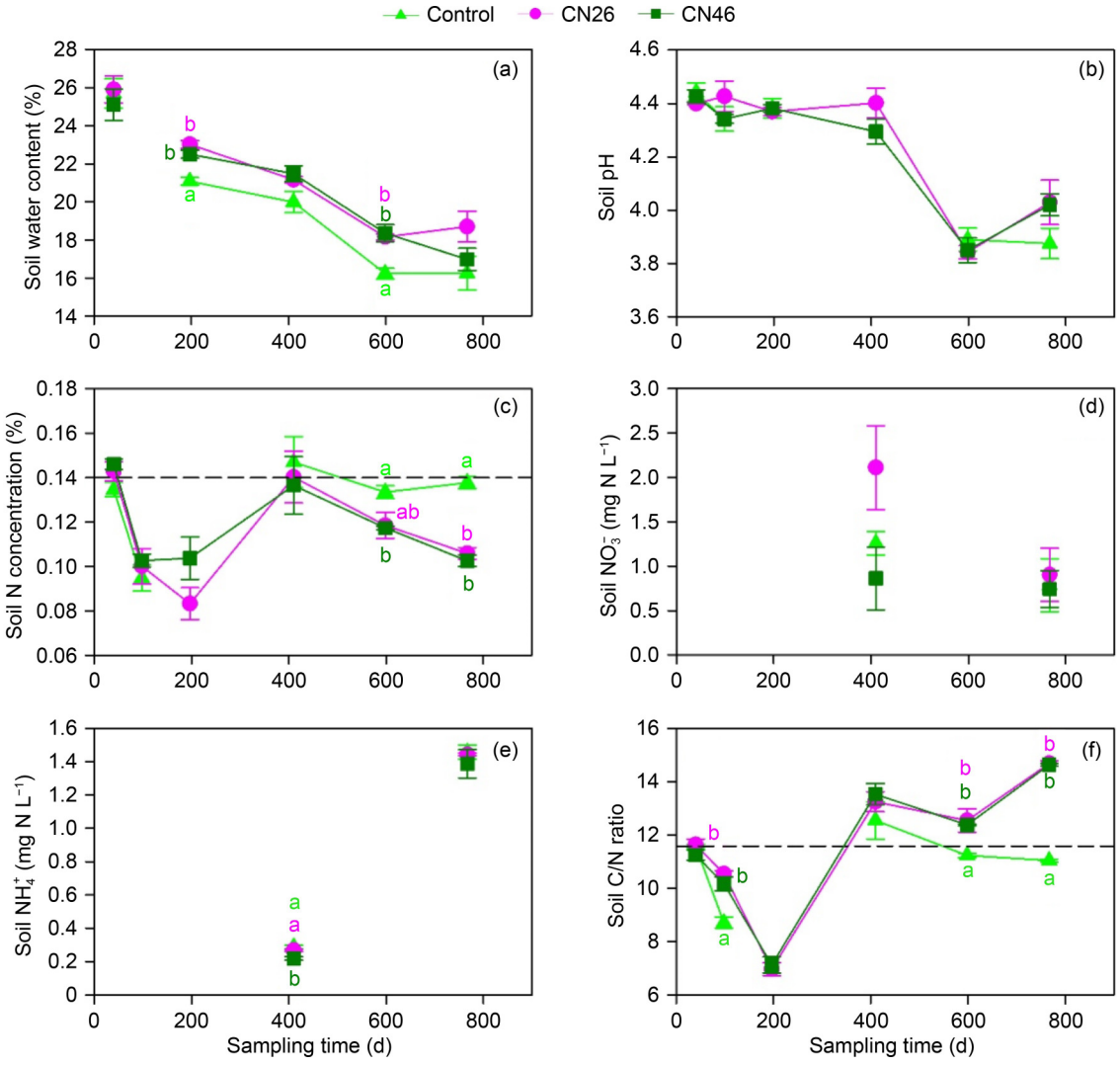


Fig. 6 Changes in soil chemical properties during two years of root litter decomposition, showing (a) soil water content, (b) soil pH, (c) total soil N, (d) soil nitrate (NO_3^-), (e) soil ammonium (NH_4^+), and (f) soil C/N ratio at two, four or six time points (40, 98, 197, 410, 598 and 767 days since the start of the experiment). CN26 (pink circles) indicates high-quality litter with a C/N ratio of 26.24; and CN46 (green squares) indicates low-quality litter with a C/N ratio of 46.24. Symbols and error bars represent means and standard errors for *n*= 4 per sampling point and root litter type. Lower-case letters indicate significant differences between litter types and/or controls, where light green letters represent controls, purple letters represent CN26 and dark green letters represent CN46.

gregate fraction under the low-quality litter at the end of the study (Table S1, Fig. 5b) coincided with greater release of C from low-quality litter after 600 days (Fig. 1f), suggesting that litter quality might have an indirect influence on macroaggregate formation through the timing of C release during decomposition. Hence, fine root litter quality had transient effects on macroaggregate formation during two years of decomposition, but these effects alone do not explain the differences in soil C storage efficiency. Given the importance of binding agents for aggregate formation (Six et al., 2000), C derived from other root products, such as exudates and mucilage, is likely to be more important for aggregate formation and stability (Dijkstra et al., 2021) than C released during decomposition.

4.3 Fine root litter quality has negligible effects on soil physicochemical properties

We expected that litter quality would affect soil chemical properties during decomposition, and that changes in soil water content, pH, and nitrogen status would contribute to differences in litter-derived C storage efficiency. However, contrary to expectations, root litter quality did not influence soil water content, soil pH or total soil N, and only had a transient effect on soil ammonium-N concentrations (Fig. 6), which suggests that either the duration of our study or the differences in litter chemistry were insufficient to alter these soil properties. The strong fluctuations in soil pH throughout the study (Fig. 6b) are likely due to leaching of colloids and

cations (particularly calcium and magnesium) by the addition of purified water (Lopez-Sangil et al., 2013). The substantial decline in soil pH after 600 days could have contributed to the low soil microbial diversity at this sampling time point (Fierer and Jackson, 2006) but there was no indication that decomposition or C storage were reduced by the decline in soil pH. Although the increase in litter N concentrations after the first 97 days (Fig. 1b) indicates N-immobilisation, the soils at the study site are N-rich (Lu et al., 2015, 2018). Total soil N concentrations are therefore unlikely to be affected by the minor differences in root litter C inputs. The high concentrations of soil ammonium-N under high-quality root litter after 400 days could indicate that litter quality influences N mineralization, but more frequent measurements of soil mineral N are needed to assess this. Thus, in our study root litter quality was the primary driver of differences in decay rates and soil C storage efficiency.

5 Conclusions

Our results reveal that differences in fine root litter quality mediate soil C storage during decomposition in subtropical forest soils in China. We found that litter quality, represented by the C/N ratio, influenced litter decay rates and the quantity of root litter-derived C stored in soils. High-quality litter decomposed faster during the early stages of decomposition (the first year of the study) but low-quality litter decomposed faster at the mid-stages (the second year of the study), resulting in similar litter mass loss by the end of the study. These findings suggest that short-term studies are unlikely to capture the complex dynamics of root litter decomposition and may produce erroneous decay rates. Despite the differences in the patterns of decomposition, C storage efficiency at the end of the experiment was greater under high-quality root litter and more litter-derived C was stored in soils compared to low-quality root litter, which indicates that microbial turnover of labile compounds in decomposing roots contribute to soil C storage. Although we found little evidence to suggest that litter quality promotes soil C storage via aggregate formation, we demonstrated that the decomposition of high-quality litter and soil C storage under low quality litter were both related to microbial diversity, which merits further attention. Thus, our study sheds new light on root litter decomposition in subtropical forests, and suggests that future work examining interactions between root litter quality, microbial communities and soil C storage efficiency could provide novel insights into the importance of decomposing roots for soil C storage.

Competing interests

The authors declare that they have no conflict of interest.

Author contributions

SX designed this study. SX and FLS did the experiments. SX analyzed the data and depicted the figures. SX and EJS wrote the paper. All authors contributed to interpretation and comment on the details of the manuscript drafts.

Data availability statement

The data set underpinning the results will be made available after acceptance.

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Electronic supplementary material

Supplementary material is available in the online version of this article at https://doi.org/10.1007/s42832-023-0182-6 and is accessible for authorized users.

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