Plant Soil
https://doi.org/10.1007/s11104-025-07219-7

RESEARCH ARTICLE

Physical rather than chemical protection determines soil organic carbon accumulation in a subtropical Chinese fir plantation treated by litter manipulation

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Received: 9 November 2024 / Accepted: 8 January 2025

Abstract
Background and aim Soil organic carbon (SOC) is crucial for soil fertility and combating climate change, which may be regulated by aboveground litter input. However, how SOC accumulation responds to litter manipulation remains unclear.
Methods In this study, we conducted a 7-year litter manipulation experiment, including litter addition, litter removal, and control treatments. We used an elemental analyzer, along with measurements of bulk density, to estimate SOC stock, the wet-sieving method to analyze soil aggregate distribution, amino sugar content to calculate microbial necromass carbon (C) in aggregates, and a Fourier transform midinfrared spectrometer (FIRT) to determine the SOC chemical functional groups along a 60 cm profile in a Chinese fir (*Cunninghamia lanceolata*) plantation.
Results We found that litter addition significantly increased SOC stock by 44.59% in the 0–10 cm layer, while litter removal had no effect on SOC stock. Litter addition increased the proportion of macroaggregates (> 2 mm) and the geometric mean diameter (GMD). Moreover, litter addition increased the bacterial necromass C in macroaggregates, which was

Responsible Editor: Tida Ge.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s11104-025-07219-7.

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Published online: 20 January 2025

positively correlated with SOC. SOC stock in topsoil was explained by GMD and fractal dimension, which might increase the protection for bacterial necromass C. However, litter addition decreased the chemical stability of SOC.

Conclusion Long-term C input increased topsoil SOC accumulation by strengthening the physical protection of aggregates rather than by the chemical protection of SOC functional groups in this Chinese fir plantation, particularly through the accumulation of bacterial necromass C in macroaggregates.

Keywords Soil organic carbon · Aggregate protection · Chemical stability · Microbial necromass carbon · Litter manipulation · Soil profile

Introduction

Soil organic carbon (SOC) is essential in mitigating climate change, as the SOC pool (1550 Pg C) is significantly larger than the vegetation carbon (C) pool (550 Pg C) and the atmospheric C pool (780 Pg C) (Antony et al. 2022; Lal 2008). Consequently, even minor shifts in SOC pool will substantially affect the fluctuations of atmospheric carbon dioxide (CO_2) (Lal 2023; Paustian et al. 2016). As an important component of global forests, subtropical forests play a key role in the global C cycle, influencing C balance and climate change (Fang et al. 2015). Aboveground litter serves as the main source of SOC in forest ecosystems, and its decomposition process regulates SOC accumulation and cycling (Klein et al. 2016; Wilcke et al. 2024). However, the link between aboveground litter input and SOC accumulation remains unclear, as previous studies observed positive, negative, or neutral effects of aboveground litter input on SOC stock (Fang et al. 2015; Liu et al. 2021; Zhao et al. 2023). Moreover, the emphasis in most studies was on topsoil, often disregarding the depth-related variation within the soil profile (Lajtha et al. 2014; Zhang et al. 2023b). Therefore, further studies are needed to explore the mechanisms of litter manipulation on SOC accumulation along the soil profile.

SOC accumulation is regulated by abiotic mechanisms, mainly through physical protection of aggregates and chemical preservation of SOC functional groups. The physical protection of soil aggregates can isolate SOC from microorganisms and their secreted enzymes (Krause et al. 2020). Macroaggregates enhance soil physical structure stability by consolidating microaggregates and organic matter, forming a stable multilayer structure (Schweizer et al. 2019; Tan et al. 2024; Xu et al. 2022). After the macroaggregates break up, the microaggregates can further stabilize SOC in them (Tan et al. 2024). The ionic bonds in the microaggregates reduce the decomposition rate of SOC by binding SOC with minerals to form stable complexes (Galicia-Andrés et al. 2021). Microbial necromass C (MNC) accounts for a large proportion of SOC and also can stabilize for a long time as MNC is usually protected by soil aggregates (Liang et al. 2017; Zhao et al. 2023).

The spatial heterogeneity of different aggregates creates different microenvironments, such as porosity, nutrient content, and oxygen availability, which will affect microbial growth, metabolism, and necromass accumulation within aggregates (Zhao et al. 2023). Functional specificity results in distinct differentiation patterns of fungal and bacterial necromass within soil aggregates (Xu et al. 2022). Fungi tend to occur more frequently in macroaggregates, whereas bacteria are more abundant in microaggregates (Liang et al. 2017; Zhao et al. 2023). Moreover, MNC is sensitive to changes in environmental conditions, on which the aboveground litter input will impose by altering the soil microclimate (e.g., temperature and humidity) and affecting the distribution of microbial communities (Murugan et al. 2019; Xu et al. 2022). However, it is still unclear how the stability of aggregates and their internal microbial necromass regulate SOC accumulation in response to litter input.

The chemical structure and stability of SOC are also important factors influencing SOC accumulation, as they indicate the degree of difficulty or ease of SOC decomposition (Stone and Plante 2015). SOC is mainly composed of C atoms and other groups of atoms, and the complexity of its chemical structure and the strength of chemical bonds determine the stability of SOC (Lei et al. 2023; Schulten and Schnitzer 1997). For example, aliphatic C, composed of lipids and free fats, and aromatic C, composed of lignin, are relatively stable in soil due to their complex chemical

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structure, while relatively simple in structure, polysaccharide C and phenolic C are easily decomposed by microorganisms (Calderón et al. 2013; Six et al. 2002). The chemical composition of SOC varies largely with ecosystems, climate conditions (e.g., temperature and precipitation), and management practices (Lajtha et al. 2014; Lei et al. 2023; Shen et al. 2018). For example, in subtropical forests with high temperature and humidity, the environment has different impacts on microbial activities and the rates of chemical reactions compared to other regions, resulting in various SOC decomposition and stability in these ecosystems (Galicia-Andrés et al. 2021; Pallasser et al. 2013). Aboveground litter is considered to be an unstable C source, which brings additional labile C input to soil (Shen et al. 2018). Additionally, increased microbial activities by aboveground litter input may stimulate the decomposition of soil labile C, introducing more uncertainty for SOC accumulation (Liu et al. 2021). Thus, how aboveground litter affects the chemical stability of SOC needs further elucidation.

The Chinese fir (*Cunninghamia lanceolata*) is a main afforestation tree species with significant C sink function and plantations of Chinese fir are widely distributed in the subtropical area of south China (Chen et al. 2015). Both physical protection through isolating microorganism attack and chemical protection through adsorbing are the key mechanisms in SOC accumulation. Some previous studies found that physical protection of aggregates was more important for SOC accumulation than inherent chemical stability, which mainly occurs in the surface layer of litter (Dungait et al. 2012; Schmidt et al. 2011; Witzgall et al. 2021). The relative contributions of physical and chemical protection may vary with soil depth due to the changes in soil structure, nutrients, and microbial properties along the profile (Wang et al. 2024b). Particularly, how aboveground litter input influences the physical and chemical protection and their relative contributions to SOC accumulation along the profile remains underexplored. In this study, we explored SOC accumulation along a 60 cm soil profile and the underlying protection mechanisms as affected by litter management in a 7-year litter manipulation experiment in a subtropical Chinese fir plantation. We hypothesized that: 1) litter addition increases whereas litter removal decreases aggregate stability, macroaggregate SOC, and MNC; 2) litter addition increases the input of labile C and the decomposability of SOC; and 3) physical protection rather than chemical structure stability results in SOC accumulation in the subtropical region in China with abundant precipitation and high temperature, but this pattern decouples with soil depth.

Materials and methods

Study site

This study was performed at the long-term field station, Qianyanzhou Ecological Station, in Taihe County, Jiangxi Province, China (115°03′29″ E, 26°44′29″ N). It had a typical subtropical monsoon climate, with average annual temperature and precipitation of 18.1 °C and 1610 mm, respectively. The soil was a typic Ultisol, weathered from red sandstone, gravel or mudstone and river alluvial (Wang et al. 2012). The study site was a second-generation Chinese fir plantation established in 1998. The average height and Diameter at breast height (DBH) of Chinese fir were 13.6 m and 11.4 cm, respectively. The stand density was 1278 hm^{-2} and the canopy density was around 76%. The understory vegetation mainly consisted of shrubs, such as *Ilex pubescens*, *Gardenia jasminoides*, *Callicarpa bodinieri* Levl., and *Adinandra millettii*, and herbs, such as *Dicranopteris linearis*, *Woodwardia japonica*, *Lophatherum gracile*, *Smilax china* L., and *Smilax glabra* (Liu et al. 2021).

Experimental design and sampling

The litter manipulation experiment was established in 2013, using a completely randomized block design with three treatments (control, litter addition, and litter removal) and three replicates for each treatment. The aboveground litter from the litter removal plot was removed and then placed in the litter addition plot, resulting in two litter manipulation treatments in addition to the control (original litter): litter addition (doubled litter) and litter removal (no litter). Each plot was 15 m × 15 m, with more than 10 m buffer zone between plots. Litter manipulation was conducted monthly over the entire year to avoid the effect of litter input. The ambient aboveground litter at the site was approximately 1.0 t ha^{-1} yr^{-1}.

Sampling was conducted in June 2019 after 7 years of treatments. Five soil profiles of 60 cm in length, width, and height were excavated in each plot using a shovel. The original soil in the four soil layers (0–10, 10–20, 20–40, and 40–60 cm, respectively) were collected with an aluminum box. Then, the visible gravels, roots, and other impurities were removed and soil was gently broken into < 8 mm along the natural fissures of the soil by hand. The soil samples of each layer from the 5 soil profiles in each plot were then mixed to form a composite soil sample. In total, 36 soil samples (3 treatments × 4 soil layers × 3 replicates) were analyzed for SOC content and physical and chemical properties.

Soil general physical and chemical properties

Bulk density (BD) was measured using a 100 cm^3 cutting ring. Soil pH was measured by a pH meter (soil: water is 1: 2.5). Dissolved organic C (DOC) was extracted with water (soil: water is 1: 10) and its content was analyzed by a total organic C analyzer (multiN/C 2100, Analytik Jena, Germany). Total nitrogen (N) and total phosphorus (P) were digested with H_2SO_4-$HClO_4$. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were extracted with 2 M KCl and available P were extracted with HCl-NH_4F. The contents of total and available nutrients were analyzed with a continuous flow autoanalyzer (Smartchem, AMS Alliance, Italy).

SOC content was determined using the H_2SO_4-$K_2Cr_2O_7$ oxidation method and SOC stock was calculated by:

$$\text{SOC stock} = \sum_{i=1}^{n} (Ci \times BDi \times Di)/10 \quad (1)$$

where Ci is SOC content of each soil layer (g kg^{-1}); BDi is the bulk density of each soil layer (g cm^{-3}); and Di is the depth of each soil layer (cm).

Soil aggregate fractionation

The fractions of soil aggregates were determined using the wet-sieving method (Dorodnikov et al. 2009; Fang et al. 2016). Soil aggregates with the following four particle sizes were obtained: (i) < 0.053 mm, (ii) 0.053–0.25 mm, (iii) 0.25–2 mm, and (iv) > 2 mm. The sub-samples of different fractions were used for SOC and MNC analyses.

Mean Weight Diameter (MWD), Geometric Mean Diameter (GMD), and fractal dimension (D) were used to evaluate the stability characteristics of aggregates (Cao et al. 2021). The specific formulae were as follows:

$$MWD = \sum_{1}^{n+1} \frac{r_{i-1} + r_i}{2} \times m_i \quad (2)$$

$$GMD = EXP(\sum_{1}^{n} \frac{Wi}{M} \lg \overline{R_i}) \quad (3)$$

$$\lg \frac{m\left(r \le \overline{R_i}\right)}{M_i} = (3 - D)\lg \frac{\overline{R_i}}{R_{max}} \quad (4)$$

where m_i is the percentage of particle mass of i-sized aggregates (%); r_i is the mesh size of sieve i (mm); n is the number of sieves; M is the total mass of the sample (g); W_i is the mass of i aggregate size (g); R_i is the mean diameter of i aggregate size (mm); m ($r \le \overline{R_i}$) is the mass of aggregates with particle size less than $\overline{R_i}$ (g); and R_{max} is the maximum particle size of the aggregates (mm).

Microbial necromass C determination

MNC contents were calculated according to the amino sugars determined by gas chromatography (Zhang and Amelung 1996). In detail, a dry soil sample of 0.5 g was hydrolyzed with 10 mL of 6 M HCl, followed by the addition of 100 μL of inositol. After mixing, the solution was subjected to rotary evaporation and filtration. pH of the solution was then adjusted to a range of 6.6 to 6.8 and centrifuged. Next, a second rotary evaporation was performed, followed by nitrogen-assisted drying. Then, 100 μL of N-methylglucosamine was added, and the sample underwent freeze-drying. Finally, 300 μL of derivatization reagent was added for derivatization. The derivatives were analyzed using a gas chromatograph (Shimadzu Corporation, Japan) equipped with a capillary column (25 m in length × 0.25 mm in diameter × 0.25 μm in thickness). Inositol was used as an internal standard for quantifying the content of each amino sugar, including glucosamine (GluN), galactosamine (GalN), and muramic acid (MurN).

We used the GluN and MurN contents to estimate the fungal (F-MNC) and bacterial MNC (B-MNC), and total MNC (T-MNC) is the sum of F-MNC and B-MNC (Appuhn et al. 2006):

$$\mathrm{F\ MNC} = \mathrm{GluN} - 2 \times \mathrm{MurN} \times \left(\frac{179.2}{251.2}\right) \times 9 \quad (5)$$

$$\mathrm{B\ MNC} = \mathrm{MurN} \times 45 \quad (6)$$

$$\mathrm{T\ MNC} = \mathrm{F\ MNC} + \mathrm{B\ MNC} \quad (7)$$

where 179.2 and 251.2 are the molecular weights of GluN and MurN, respectively; 9 is the conversion value of GluN to F-MNC; and 45 is the conversion value of MurN to B-MNC (Liang et al. 2019).

Chemical structure and stability of SOC

The chemical structure of SOC was analyzed by a Fourier transform midinfrared spectrometer (FIRT) spectrometer (Spectrum Two, Perkin Elmer, Waltham, MA, USA). A dry soil sample was mixed with KBr at a ratio of 1:100 and then ground into powder with an agate mortar. The mixed material was pressed into thin slices for scanning under the range of 4000–400 cm^{-1} for 32 times at a 4 cm^{-1} resolution with KBr as background. The FTIR spectrum characteristic peaks of SOC functional groups were classified according to the attribution of different infrared spectral absorption peaks. Different characteristic peaks represented different functional groups: 3632 cm^{-1} for alcohol and phenol C–OH, 2920 and 2851 cm^{-1} for aliphatic C-H, 1630, 930 and 840 cm^{-1} for aromatic C = C, and 1030 cm^{-1} for polysaccharide C-O (Calderón et al. 2013; Lei et al. 2023; Zhou et al. 2023).

SOC decomposition degree (SD) and chemical stability (SS) represented SOC chemical stability, which were calculated by the areas of specific wavelength peaks (Margenot et al. 2015):

$$SD = \mathrm{aromatic\ C} = \mathrm{C}/\mathrm{aliphatic\ C} - \mathrm{H} \quad (8)$$

$$SS = (\mathrm{aromatic\ C} = \mathrm{C} + \mathrm{aliphatic\ C} - \mathrm{H})/(\mathrm{alcohols\ and\ phenols\ C} - \mathrm{OH} + \mathrm{polysaccharide\ C} - \mathrm{O}) \quad (9)$$

Statistical analysis

Normality and homogeneity of variance of all data were tested before analyzed. Two-way ANOVA was used to compare the effects of litter manipulation, soil depth, and their interaction on SOC in bulk and aggregate soil, aggregate stability, MNC in aggregates, and SOC chemical structure and stability. Tukey's test was used to test the significance of all the variables among the three litter treatments or four soil depths ($P < 0.05$). Pearson analysis was used to examine the relationship between SOC in bulk and aggregate soil and aggregate stability, MNC in aggregates, and SOC chemical structure and stability. Random forest (RF) analysis was used to identify the major predictors of SOC. The significance of each predictor to the response variable was assessed using the "rfPermute" package in R 4.3.2. All data were analyzed by IBM SPSS Statistics 22.0 for Windows (at the significance level of $P = 0.05$). All graphs were plotted with Origin software (version 2021).

Results

SOC and soil physicochemical properties

Litter addition notably increased both SOC content and stock, but this impact diminished at deeper soil layers. In contrast, litter removal did not change SOC content and stock at all soil depths (Fig. 1). Additionally, litter addition enhanced total N and total P at both 0–10 and 10–20 cm soil depths, as well as DOC across all soil layers (Table S1). Litter removal decreased soil pH, NH_4^+-N, and NO_3^--N at 0–10 cm soil depth. SOC content and these physicochemical indices decreased with increasing soil depths (Fig. 1, Tables S1 and S2).

Soil aggregates and their SOC and microbial necromass C

Litter addition significantly increased and decreased the proportion of > 2 mm and < 0.053 mm aggregates at the 0–10 cm soil depth, respectively (Fig. 2). In contrast, litter removal significantly reduced the proportion

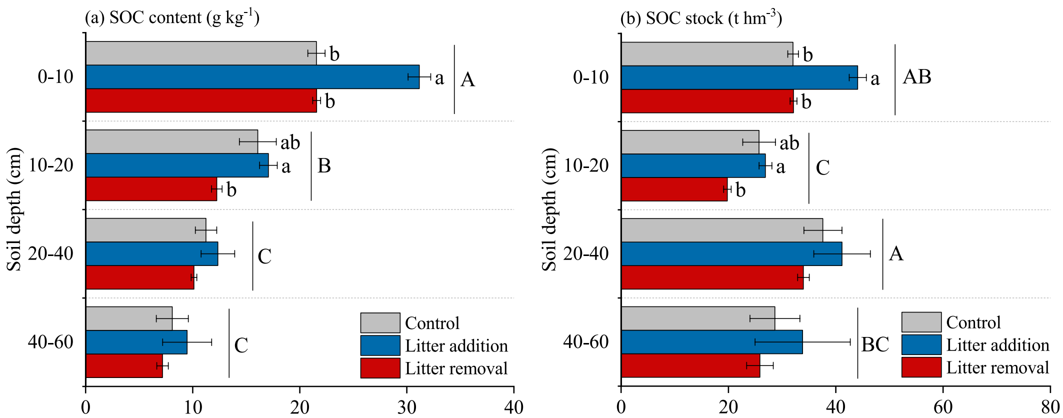

Fig. 1 Soil organic carbon content (**a**) and stock (**b**) along a 60 cm profile under different litter manipulations in a Chinese fir plantation. Values are means ± standard errors ($n=3$). Different lowercase letters indicate significant differences among three litter treatments within the same soil depth and different uppercase letters indicate significant differences of the averages of three litter treatments among the four soil depths ($P<0.05$)

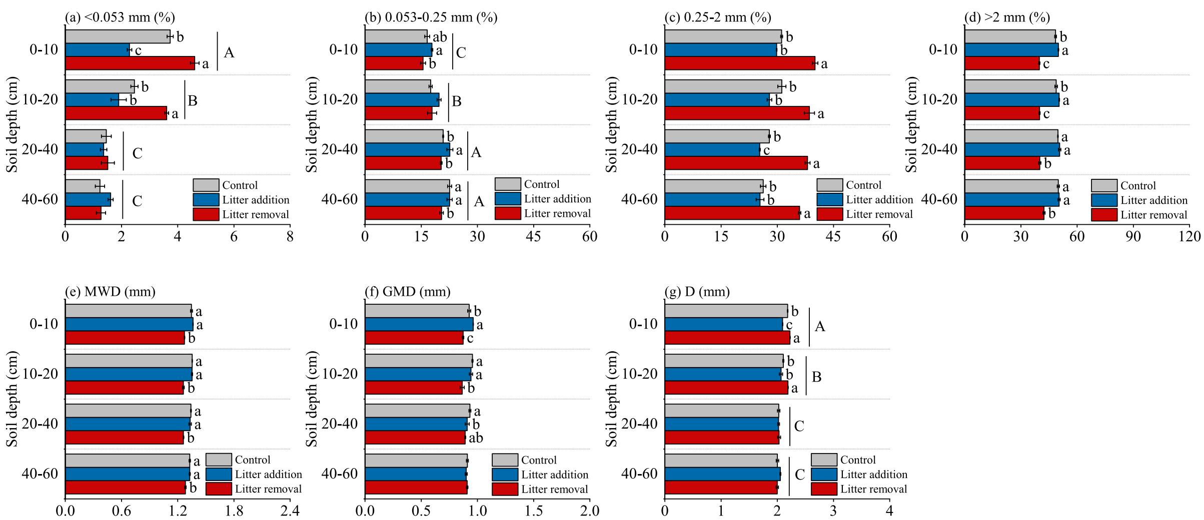

Fig. 2 The composition (**a-d**) and stability (**e-g**) of aggregates along a 60 cm profile under different litter manipulations in a Chinese fir plantation. Values are means ± standard errors ($n=3$). Different lowercase letters indicate significant differences among three litter treatments within the same soil depth and different uppercase letters indicate significant differences of the averages of three litter treatments among the four soil depths ($P<0.05$). MWD: mean weight diameter, GMD: geometric mean diameter, D: fractal dimension

of > 2 mm aggregates at both 0–10 and 10–20 cm soil layers. Different sizes of soil aggregates exhibited different patterns along the soil profile (Fig. 2). Additionally, GMD significantly increased while D decreased with litter addition in the 0–10 cm soil layer. In contrast, litter removal exhibited the opposite effects on GMD and D (Fig. 2). Overall, MWD, GMD, and D in deeper soil layers were less sensitive to litter addition or removal (Fig. 2, Table S1).

As for SOC content in soil aggregates, SOC content in the > 2 mm aggregates increased but that in the < 0.053 mm aggregates decreased with litter addition at the 0–10 cm soil layer (Fig. 3). Litter removal did not significantly affect SOC content in any aggregate fractions (Fig. 3). In addition, SOC content in all

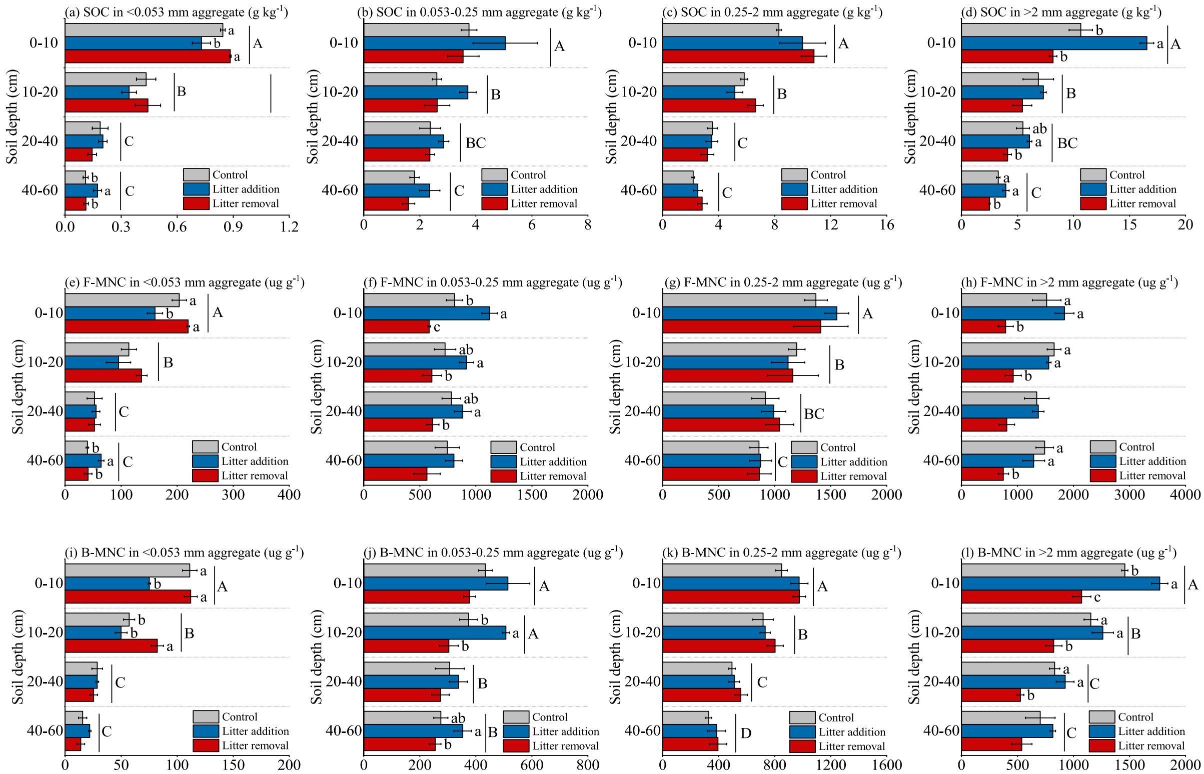

Fig. 3 Soil organic carbon content (**a**-**d**), fungal (**e**-**h**) and bacterial (i-l) microbial necromass carbon (MNC) in different aggregates along a 60 cm soil profile under different litter manipulations in a Chinese fir plantation. Values are means ± standard errors ($n = 3$). Different lowercase letters indicate significant differences among three litter treatments within the same soil depth and different uppercase letters indicate significant differences of the averages of three litter treatments among four soil depths ($P < 0.05$). F-MNC: fungal necromass carbon, B-MNC: bacterial necromass carbon

the four aggregate fractions declined along the soil profile (Fig. 3).

Litter manipulation had significant impacts on MNC in different aggregates. Specifically, litter addition significantly enhanced MurN, T-MNC, and B-MNC in the > 2 mm aggregates, while it decreased Ass, GluN, MurN, T-MNC, F-MNC, and B-MNC in the < 0.053 mm aggregates at the 0–10 cm soil depth (Figs. 3, S1 and S2). Litter removal increased MurN and B-MNC in the < 0.053 mm aggregates at the 10–20 cm soil depth, but decreased Ass and MNC in the > 2 mm aggregates across all soil layers. Additionally, MNC variables tended to decrease along the soil profile except for F-MNC in the > 2 mm aggregates (Figs. 3, S1 and S2, Tables S1).

SOC chemical structure and stability

Litter addition and soil depth significantly affected the chemical structures of SOC and SS, while their interaction influenced only the phenolic C-O group (Fig. 4 and Table S1). In detail, litter addition increased the proportions of phenol C-O and polysaccharide C-O at the 0–10 cm soil depth, while decreasing the proportions of aromatic C = C and aliphatic C-H at the soil layers above 20 cm. Notably, litter removal did not alter the chemical structures of SOC. Litter addition reduced soil chemical stability at the soil layers above 20 cm. Furthermore, both phenol C-O and polysaccharide C-O proportions declined with increasing soil depths, whereas aromatic C = C and aliphatic C-H displayed the opposite trend (Fig. 4 and Table S1).

Contributions of physical versus chemical protection to SOC accumulation

Results of the RF model revealed that changes in SOC at the 0–10 cm soil depth were primarily attributed to

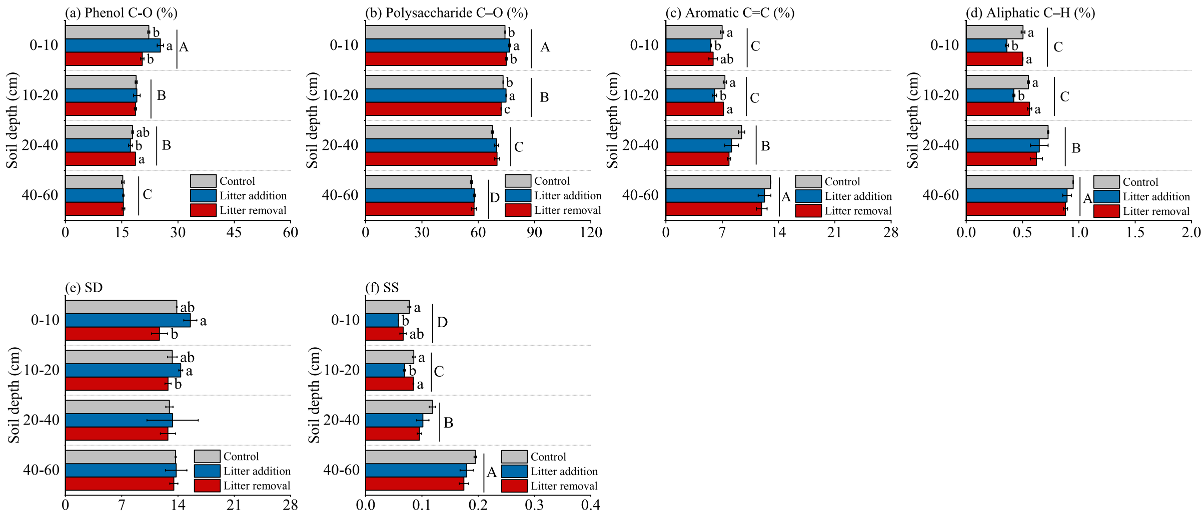

Fig. 4 Soil organic carbon chemical structure (**a-d**) and stability (**e-f**) along a 60 cm profile under different litter manipulations in a Chinese fir plantation. Values are means ± standard errors ($n=3$). Different lowercase letters indicate significant differences among three litter treatments within the same soil depth and different uppercase letters indicate significant differences of the averages of three litter treatments among four soil depths ($P<0.05$). SD: soil organic carbon decomposition degree, SS: soil organic carbon chemical stability

the physical protection through aggregates although some of the chemical structure and stability also showed significant correlations with SOC stock (Figs. 5 and S4, Table S4). SOC at the 0–10 cm soil depth was positively correlated with GMD, but negatively correlated with the proportion of < 0.053 mm aggregates and D. Phenol C-O, polysaccharide C-O, and SD all had positive relationship with SOC at this topsoil layer unlike aliphatic C-H, whose relationship with SOC stock was negative (Fig. 5, Table S4). At the 10–20 cm depth, polysaccharide C-O emerged as the main factor contributing to SOC accumulation, with a significantly positive correlation with SOC (Figs. 5 and S4, Table S4). Conversely, at the 40–60 cm depth, SOC was found to be controlled jointly by physical and chemical mechanisms: D, the proportion of < 0.053 mm aggregates, and aromatic C=C (Figs. 5 and S4, Table S4).

SOC accumulation was primarily dominated by SOC in > 2 mm aggregates at the 0–10 cm depth (Fig. S3). SOC in the > 2 mm aggregates at the 0–10 cm soil depth was positively correlated with B-MNC in the same aggregate size. Additionally, SOC in the > 2 mm aggregates at the 20–40 cm soil depth was positively correlated with T-MNC, F-MNC, and B-MNC within the same size fraction, as well as with B-MNC in the > 2 mm aggregate at the 40–60 cm soil depth (Fig. S4 and Table S4).

Discussion

This research investigated how litter input influences SOC accumulation and the underlying mechanisms along a 60 cm soil profile in a Chinese fir plantation. We found that changes in litter input could significantly affect SOC accumulation. Moreover, we found that physical rather than chemical protection regulated topsoil SOC accumulation in this ecosystem treated by a 7-year litter manipulation.

Effects of litter manipulation on aggregate stability

The study supports our hypothesis that litter addition increases while litter removal decreases soil aggregate stability. This is because litter addition increased GMD and the proportion of > 2 mm aggregates at the 0–10 cm soil layer, while litter removal exerted the opposite effects (Fig. 2). Litter decomposition produces polysaccharides and humus, which act as “binding agents” to help soil particles aggregate into more stable aggregates (Blanco-Canqui and Lal 2004; Liu et al. 2014). As a result, reduced litter input would weaken the binding effects between soil particles, leading to reduce aggregate stability (Blanco-Canqui and Lal 2004; Liu et al. 2014). Moreover, the presence or absence of aboveground litter also affects soil erosion resistance, thereby

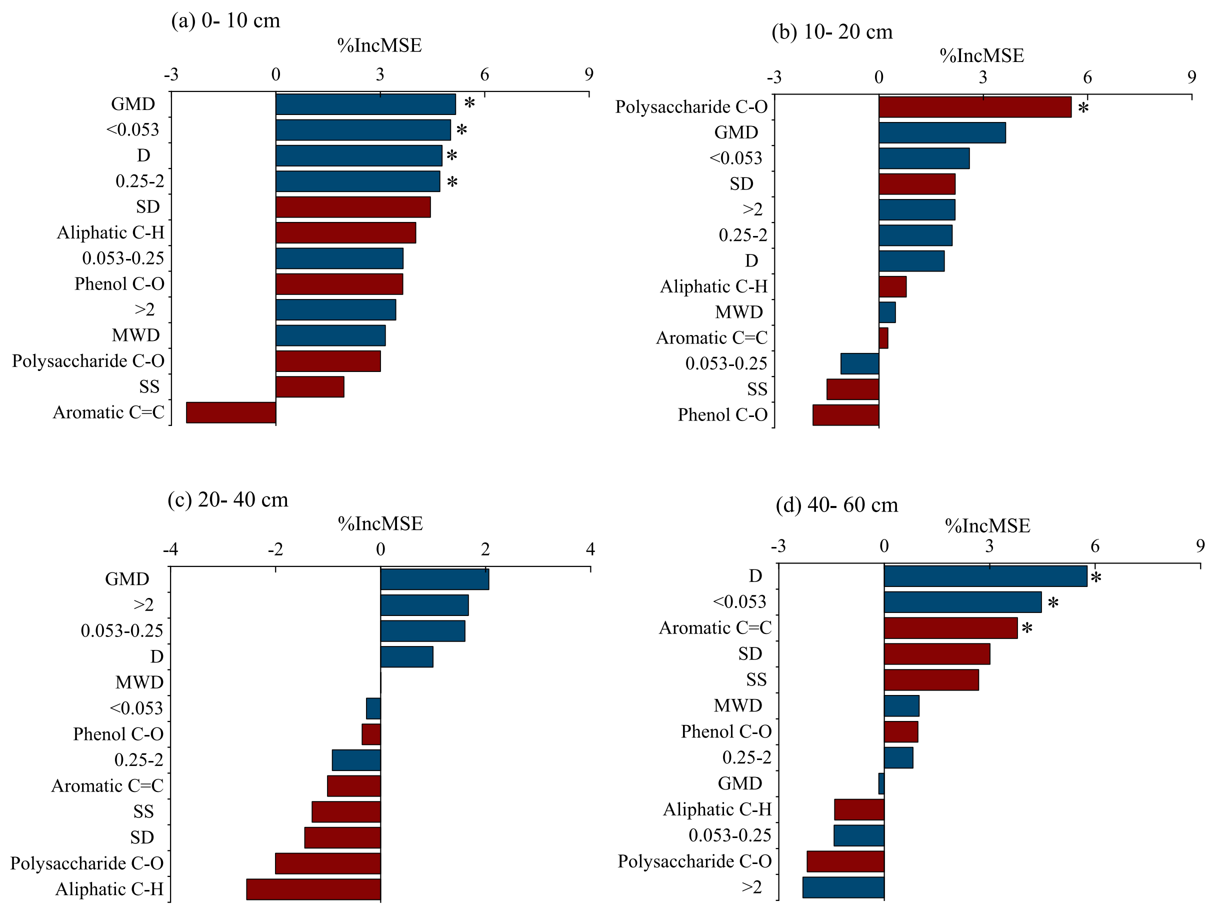

Fig. 5 Relative importance of physical versus chemical protection for predicting SOC from the random forest model. The blue and the red columns indicate physical compositions and stability and indicate chemical structure and stability, respectively. <0.053: <0.053 mm aggregate proportion, 0.053–0.25: 0.053–0.25 mm aggregate proportion, 0.25–2: 0.25–2 mm aggregate proportion, >2: >2 mm aggregate proportion, MWD: mean weight diameter, GMD: geometric mean diameter, D: fractal dimension, SD: soil organic carbon chemical stability, SS: soil organic carbon decomposition degree, IncMSE: increase in mean square error (%). * $P<0.05$

influencing aggregate stability (Saha et al. 2024). The presence of aboveground litter safeguards the ground from direct exposure to the force of falling rain (Saha et al. 2024). In contrast, without the protection of aboveground litter, the soil surface becomes more prone to fragmentation, resulting in loose aggregate structure and reduced stability (Ozlu and Arriaga 2024). In addition, the formation of arbuscular mycorrhizal fungi (AMF) also enhances the stability of aggregates since AMF promotes soil aggregation through hyphal entanglement and the production of glomalin-related soil proteins (GRSP) (Wei et al. 2019). GRSP is highly viscous and difficult to decompose. It can bond soil particles together, promoting the formation and stability of soil aggregates. The mycelia of AMF can be wound around the surface of soil particles and thus play a role in physical fixation and connection of soil particles, increasing the stability of soil aggregates. A previous study reported that litter addition increased the abundance of AMF because exogenous C input could increase the SOC content, providing more energy and substrate for the growth and activity of AMF (Johnson 2010). Thus, aggregate stability increases with the amount of aboveground litter.

Effects of litter manipulation on SOC and MNC in aggregate fractions

Litter addition increased SOC in the >2 mm aggregates, while litter removal had no effect on SOC across all sizes of aggregates at the 0–10 cm soil layer (Fig. 3). This suggests that SOC in aggregates responds more strongly to litter addition than removal in the topsoil. After litter addition, a substantial quantity of organic C enters the soil through decomposition (Liu et al. 2021). Macroaggregates, with larger pore structures, can retain more litter decomposition products and microbial metabolites compared to microaggregates (Zhao et al. 2023). These organic materials become protected within the aggregates, increasing SOC in macroaggregates (Luo et al. 2024). Conversely, no effect of litter removal on SOC in aggregates was observed (Fig. 3), even though the soil aggregate stability was reduced by litter removal (Fig. 2). This may be due to other organic C sources, such as root exudates, plant residues, and microbial residues, which continue to contribute C to aggregates even after litter removal, thereby slowing SOC reduction (Pausch and Kuzyakov 2018). Additionally, our findings indicated that SOC in macroaggregates contributes the most to SOC in bulk soil (Fig. 3), as aboveground litter input enhanced the proportion of macroaggregates (> 2 mm) (Fig. 2). According to aggregation hierarchy theory, macroaggregates contain more SOC than microaggregates (Six et al. 2002). Thus, our study highlights that the accumulation of macroaggregate C is the crucial mechanism for increasing SOC with litter addition.

In the topsoil, litter addition resulted in an increase in bacterial MNC rather than fungal MNC in the > 2 mm aggregates (Fig. 3). This suggests that litter addition promotes the formation and stabilization of bacterial necromass in macroaggregates. Aboveground litter provides rich C source for microorganisms, which is more favorable for the growth and metabolism of bacteria than fungi (Zhou et al. 2024). Our previous study supported this observation, showing that bacteria grow faster than fungi under litter addition (Table S3). Additionally, bacteria also have a high metabolic rate, enabling them to rapidly decompose and utilize simple organic compounds (Wang and Kuzyakov 2024). This rapid metabolism allows bacteria to efficiently decompose litter and other organic materials, contributing to bacterial MNC formation. Although fungi are highly effective at decomposing complex organic materials like lignin and cellulose, increasing the physical protection of soil aggregates, overall turnover rate of fungi remains slow (Wang and Kuzyakov 2024). Furthermore, we observed a significantly positive correlation between bacterial MNC and SOC in the > 2 mm aggregates (Fig. S4), reinforcing the notion that bacterial MNC, rather than fungal MNC, is key to SOC accumulation. The accumulation of bacterial MNC in macroaggregates enhances the C decomposition resistance through physical isolation, chemical binding and biological cementation, and optimizes soil structure and function (Murugan et al. 2019; Shen et al. 2018). The accumulation of bacterial MNC in macroaggregates prolongs the turnover time of C and enhances the capacity of soil as a long-term C stock, which is significance to the global C cycle (Shen et al. 2018; Zhou et al. 2023). Overall, the rise in SOC in macroaggregates is primarily regulated by the accumulation of bacterial MNC under litter addition.

Effects of litter manipulation on SOC chemical stability

In this study, litter addition increased the proportion of phenol C-O and polysaccharide C-O, while decreasing the proportion of aromatic C = O and aliphatic C-H (Fig. 4). These results indicated aboveground C input induced the SOC chemical structure transition from stable to unstable C, resulting in the reduced chemical stability of SOC (Fig. 4). Phenol C-O and polysaccharide C-O are often labile C sources due to their relatively simple chemical structure and easy use and transformation by microorganisms, while aromatic C = O and aliphatic C-H are usually characterized as resistant C sources due to their conjugated stability of chemical structure and low chemical reactivity of aliphatic groups (Lei et al. 2023; Six et al. 2002). Simple compounds in the litter are preferentially decomposed by microorganisms and incorporated into the soil, which increases the proportion of soil labile C and thereby weakens the SOC chemical stability (Shen et al. 2018). Furthermore, microbial diversity and activity are another important factor influencing the chemical stability of SOC. Aboveground litter contains a variety of organic C compounds that are easy or difficult to decompose (Wang et al. 2024a). These diverse C sources provide a rich source of nutrients for different bacteria and fungi, increasing microbial diversity and activities but decreasing the SOC stability (Angst et al. 2021; Liu et al. 2021). However, litter removal did not affect the SOC chemical structure and stability (Fig. 4). Litter removal reduces the contribution of aboveground organic matter, but the iron and aluminum oxides can form chemical bonds with functional groups of compounds (e.g., carboxyl and hydroxyl groups) on the surface of organic C molecules, which makes SOC more difficult to be decomposed by microorganisms, thereby improving its stability (Luo et al. 2024). These recalcitrant C may not change significantly in the short term due to litter removal. Thus, litter addition reduced the stability of SOC by increasing the proportion of labile C, whereas litter removal did not significantly affect SOC stability.

Key driving factors to SOC accumulation along soil profile

SOC in the topsoil was primarily determined by physical rather than chemical protection (Fig. 5). This is because the surface soil, the most active area for soil organic matter accumulation and turnover, was directly affected by litter input, root activities, and microbial metabolism (Liu et al. 2021). Additionally, C/N ratio and the content of lignin and cellulose in Chinese fir litter was high, while the content of soluble organic matter was low (Fang et al. 2021). This chemical composition causes litter to decompose slowly and provide nutrients inefficiently in the short term (Fang et al. 2021). Therefore, the change of SOC accumulation and its coupling with physicochemical protection only occurred in the topsoil after 7-year litter manipulation.

In addition, SOC accumulation is not dependent on the intrinsic chemical structure of SOC, but is primarily due to the physical protection by the aggregates that limits the decomposition of SOC (Dungait et al. 2012;

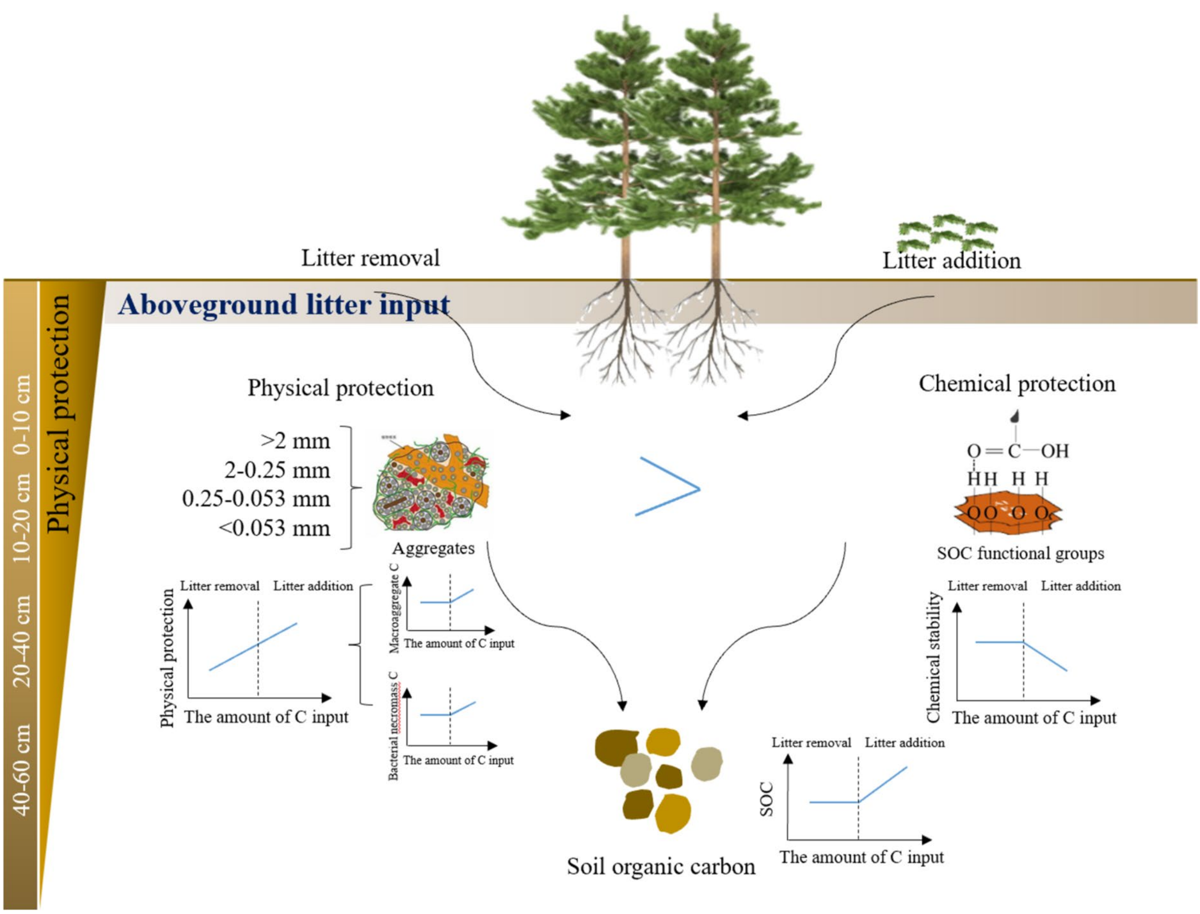

Fig. 6 Schematic diagram illustrating the impact of litter manipulation on SOC accumulation along a 60 cm soil profile and the underlying physical and chemical protection mechanisms

Schmidt et al. 2011; Witzgall et al. 2021). Surface soil organic matter interacts with soil particles (e.g., clay and silt) to form soil aggregates due to the abundant sources of organic matter (e.g., plant litter and root exudates) and rich clay minerals in warm and humid subtropical areas (Zhou et al. 2023). These aggregates enclose organic matter and physically isolate direct contact with microorganisms, thus increasing the SOC accumulation (Wang et al. 2024b). At the same time, although compounds such as carboxyl and hydroxyl groups in surface soil organic matter can form chemical bonds with iron and aluminum oxides or clay particles, these chemical bonds are easily to be dissociated by microbial secretions, pH fluctuations, or ion competition (Angst et al. 2021; Lie et al. 2023; Pallasser et al. 2013). In addition, the topsoil is exposed to larger changes in water and oxygen supply than subsoil that may accelerate the breakdown of chemical bonds (Pallasser et al. 2013). Thus, the stability of SOC relies primarily on the physical protection by aggregates rather than their chemical resistance.

The physical protective effect of aggregates diminished as soil depth increased (Figs. 5 and S4) due to several factors. i) The input of exogenous organic matter decreased significantly with soil depth (Liu et al. 2021). C input of deep soil mainly depends on organic matter permeated by deep roots, but the quality of organic matter is low and the distribution of organic matter is not uniform along the profile (Liu et al. 2019). Less input of organic matter to deep soil reduces the supply of "binding agent" needed for aggregate formation, thereby reducing the physical protection of organic carbon by aggregates (Blanco-Canqui and Lal 2004). ii) Microbes are less active in deeper soils due to lower nutrient and oxygen availability (Liu et al. 2021). Microorganisms utilize their metabolites to interact with soil minerals, forming adhesives that enhance binding (Zhang et al. 2023a). Therefore, decreased microbial activity hampers aggregate formation and stability. iii) The physical properties of deeper soils, including particle composition and texture, may differ from those of the upper layers (Abrar et al. 2020; Angst et al. 2021). Deep soils usually have fewer fine particles and binders, resulting in lower aggregate formation efficiency and weaker physical protection of SOC (Angst et al. 2021). The undisturbed properties of deep soil provide a relatively stable C stock for the global C cycle, which is reflected not only in its storage capacity, but also in its function of protecting and stabilizing existing C stocks. This stability provides an important guarantee for long-term C sequestration and mitigation of climate change.

Conclusion

By examining SOC accumulation and the controlling factors in relation to litter manipulation along a 60 cm soil profile in a subtropical Chinese fir plantation, we revealed the regulations of litter input on SOC accumulation and the driving factors for SOC accumulation. Compared with control, SOC in topsoil increased due to litter addition but was not altered by litter removal. Litter addition also enhanced aggregate stability, with the macroaggregate C being the key factor controlling SOC accumulation. Moreover, the increase in macroaggregate C was primarily attributed to a greater rise in bacterial MNC than fungal MNC. Furthermore, litter addition elevated labile C but reduced resistant C, resulting in a decreased chemical stability of SOC. Thus, the physical protection offered by aggregates, rather than chemical protection of SOC functional groups, serves as the primary mechanism for SOC accumulation in the topsoil, while their relationship weakened with increasing soil depth. Overall, long-term C input increased SOC accumulation by strengthening the physical protection of aggregates in this Chinese fir plantation, particularly through the accumulation of bacterial MNC in macroaggregates (see Fig. 6). Therefore, proper management of litter C inputs can enhance the accumulation of SOC and mitigate the global climate change.

Author contributions Qiao Liu: Writing – original draft, Visualization, Methodology, Formal analysis, Writing – review & editing, Conceptualization. Xiangjiang Liu: Formal analysis. Zhigao Liao: Investigation. Shengnan Wang: Supervision, Funding acquisition. Junjie Huang: Writing – review & editing, Methodology, Visualization. Yiqi Luo: Writing – review & editing. Lifen Jiang: Writing – review & editing. Geoff. G Wang: Writing – review & editing. Huiming Wang: Project administration, Methodology, Conceptualization. Fusheng Chen: Writing – review & editing, Visualization, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Funding This study was supported by the National Natural Science Foundation of China (grant numbers 32471851 and 32171759), Double Thousand Plan of Jiangxi Province (jxsq2023201058), and Natural Science Foundation of Jiangxi Province (20242BAB20300).

Data availability The data that support this study will be shared upon reasonable request to the corresponding author.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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