

Small straw addition enhances straw decomposition and carbon stabilized in soil aggregates over time

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ABSTRACT

Straw return as a common and effective management can improve soil carbon (C) sequestration and structure by forming aggregates and aggregate associated C. Quantifying soil aggregation and aggregate associated C is key to predicting soil organic C dynamics using testable models. The relative quantity of C stabilized in soil aggregate is an indicator that can reveal the conversion efficiency of added straw to organic C in aggregate. Straw size is known to alter litter decomposition, but how straw size impacts the dynamics of soil aggregate formation and the relative quantity of C stabilized in soil aggregate after litter addition is not well understood. We conducted an 80-day laboratory incubation with wheat straw addition of two different sizes (1–2 mm and <0.25 mm) to artificial soils of two textures (3 % clay and 7 % clay). The objectives were to examine 1) whether straw size significantly modified litter decomposition and the relative quantity of C stabilized in soil aggregates over time, and 2) what soil physical and biochemical factors determined soil aggregation and the formation of aggregate-associated C over time, such as straw size, soil texture, or enzyme activity. We found that both litter decomposition and the relative quantity of C stabilized in soil aggregates were higher in the treatment with small straw addition than those with large straw addition, but they were not significantly different between two soils with 3 % vs. 7 % clay content. This was due to increased enzyme activities caused by small straw addition, which could enhance dissolved organic C generation and stimulate microbial decomposition. Additionally, the mass proportion of macroaggregate (>2 mm) increased with time while the mass proportion of microaggregate (0.25–2 mm) decreased, suggesting that microaggregate combined to form macroaggregate (>2 mm) in the late stage of incubation (after day 17). Moreover, the relative quantity of C stabilized in microaggregate (<0.053 mm) increased linearly with dissolved organic C, whereas the relative quantity of C stabilized in macroaggregate (>2 mm) increased with the aromaticity of dissolved organic matter as measured by specific ultraviolet absorbance at 254 nm. Together, our findings suggest that straw size remarkably modifies litter decomposition and the relative quantity of C stabilized in soil aggregates by changing the quantity and quality of dissolved organic C at different decomposition stages, whereas small difference in soil clay content has no effects.

1. Introduction

Soil carbon (C) sequestration is important for mitigating global warming and preserving soil quality. Plant litter addition or straw return are very effective to replenish lost soil organic C (SOC) or to achieve SOC

accrual (Cotrufo et al., 2015; Craig et al., 2022; Sokol et al., 2019). The relative quantity of plant litter C stabilized in soil, which indicates the transformation of litter C to SOC, is a key measure for soil C sequestration. Plant inputs can be stabilized by soil aggregates of various sizes through physical isolation (Six et al., 2002) and physical-chemical

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Table 1

The percentage (%) of mineral composition of two artificial soils with different textures.

Artificial soil	Illite (g)		Quartz (g)		
	Clay-sized (<2 μm)	Silt-sized (<53 μm)	Silt-sized (<53 μm)	Sand-sized (53–250 μm)	Sand-sized (250–2000 μm)
7 % clay	7	7	37.12	38.31	10.57
3 % clay	3	3	39.69	42.43	11.89

sorption (Fulton-Smith and Cotrufo, 2019; Singh et al., 2018; Yu et al., 2015). Soil aggregation and organic C associated with aggregates vary with time and management disturbance (e.g., straw addition) (Huang et al., 2018). Since 1959, a series of theories have been proposed to explain the dynamics of soil aggregates and aggregate-protected organic C (Six et al., 2004). The aggregate hierarchy theory suggests that soil aggregates can grow overtime and then shrink again following litter addition, and changes in micro- and macro-aggregates are linked to variations in SOC levels, which in turn affect one another (Chen et al., 2022; De Gryze et al., 2006; Oades, 1984; Peng et al., 2017; Six et al., 1998). Recently, soil C models based on the aggregate hierarchy theory, such as AggModel (Segoli et al., 2013) and the Millennial model (Abramoff et al., 2018), have received attention because the SOC pools in these models are measurable, making the models testable and more accurate in predicting SOC dynamics (Dangal et al., 2022; Sohi et al., 2001). Therefore, understanding the impacts of plant litter addition on how soil aggregation and aggregate-associated organic C vary with time is important for predicting SOC dynamics.

Straw size could affect litter decomposition and then the formation of soil aggregation and aggregate-associated C (Bucka et al., 2021). In real farmland management, straw is typically shredded to a size of 2–10 cm at the centimeter level (Li et al., 2023; Meng et al., 2021; Meng et al., 2024; Wang et al., 2023a; Wang et al., 2023c). But smaller straw (0.5–1.0 cm) is recommended for cropland management (Wang et al., 2023b), given its faster decomposition rate compared to larger straw (>2 cm). Although millimeter-sized powdered straw is uncommon in farms, it is widely used in many laboratory incubation experiments because it can be evenly mixed with soil, and we can promptly observe changes in straw decomposition and soil C sequestration (Loecke and Robertson, 2009; Xu et al., 2022). Due to different specific surface areas (surface area per unit of mass), small (millimeter-sized) and large (centimeter-sized) straws have various abilities for microbial communities and mineral particles to attach (Tuomi et al., 2011), which are crucial for litter decomposition and organic C stabilization (Struecker et al., 2016). Small straws allow more microbes to colonize and accelerate the decomposition of litter due to their larger specific surface area compared to large straws (Rinkes et al., 2013). Moreover, small straws release a greater amount of soluble compounds (e.g., phenols and nutrients), which further enhances litter decomposition (Rinkes et al., 2013). Besides, small straws with a larger specific surface area can contact more minerals. This can induce particle assembly around litter, resulting in a reduction in litter decomposition as mineral-associated organic C is formed. During litter decomposition, microbes tend to preferentially utilize non-structural soluble organic compounds, such as dissolved organic matter (DOM), which can be directly adsorbed by minerals. Meanwhile, the structural litter residue can result in the formation of particulate organic matter (POM) (Cotrufo et al., 2015). POM derived from large straw can act as aggregate nucleus by providing multiple sites for mineral particles to adhere to, owing to their prominent physical structure (Witzgall et al., 2021). Small and large straws behave differently in terms of litter decomposition and the formation of aggregates and aggregate-associated organic C. However, few studies have simultaneously assessed both litter decomposition and the stabilization of litter C in aggregates after the addition of different-sized

straw. Whether returning small straw will lead to greater soil C sequestration, such as higher relative quantity of C stabilized in aggregates, remains poorly understood.

Understanding the pathways of how plant litter is transformed into aggregate protected C is crucial in predicting SOC dynamics. According to the traditional view, the chemically recalcitrant organic components of litter (e.g., lignin) persist in soil and govern the formation of SOC (Bollag et al., 1997; Shahbaz et al., 2017). In recent years, Cotrufo et al. (2015) proposed that labile components of plant litter (e.g., dissolved organic C (DOC)) could form stable SOC by sorbing on soil minerals through organo-mineral interactions during the early stages. Meanwhile, resistant litter residues would move within soil through physical transfer to create coarse POM during the late stage of decomposition. In addition, microbially mediated soil C models predict that plant inputs are firstly transferred to DOC by extracellular enzymes (Allison et al., 2010), which could stimulate microbial metabolic activity, such as CO₂ emission and microbial biomass. DOC and microbial biomass (necromass) are key precursors for the formation of stable SOC (e.g., mineral-associated organic C) (Frey et al., 2013; Lavallee et al., 2018; Liang et al., 2017). Thus, the regulation of SOC formation is dependent on DOC and enzyme activities. Although research has tested the pathway of the formation of different SOM fractions after litter addition (Fulton-Smith and Cotrufo, 2019), the relationships of soil aggregation and aggregate associated C with DOC and enzyme activity over time remain unclear.

This study aimed to examine 1) whether straw size had a significant impact on litter decomposition and the relative quantity of C stabilized in soil aggregates over time, and 2) what soil physical and biochemical factors determined soil aggregation and the formation of aggregate-protected C over time, such as straw size, soil clay content, or enzyme activity. We hypothesized that 1) adding small straw (<0.25 mm) and increasing clay content promotes soil aggregation, leading to higher relative quantity of aggregate-protected C, and 2) labile organic components (e.g., DOC) enhance the C formation in small aggregates during the early stage after litter addition, whereas structural organic components promote the C formation in large aggregates during the later stage. To reveal the temporal dynamics of soil aggregation and aggregate-associated C following litter addition, we used artificial soils with pre-determined composition and initial properties to simplify the study materials (Pronk et al., 2012).

2. Materials and methods

2.1. Laboratory incubation experiment design

To evaluate how straw size affects soil aggregation dynamics and the relative quantity of C stabilized in different aggregates, we conducted a laboratory incubation experiment with straw added to artificial soil. Using a two-factor completely randomized design, the effects of straw size and clay content on the formation of soil aggregates and aggregate-protected C in sandy soils was examined in this short-term laboratory incubation. According to Bucka et al. (2021), the experiment included preparing artificial soil, adding straw in two sizes, and adding nutrient solution. The laboratory incubation consisted of six treatments, each with three replicates: three straw additions and two soil textures. To examine soil aggregation dynamic after straw addition, we created seven batches of artificial soil for destructive sampling at seven time points (1, 4, 7, 17, 31, 52, and 80 days of incubation), generating a total of 198 samples.

Specifically, the three treatments of wheat straw addition (two straw sizes 0.1–0.2 cm, <0.25 mm, and no litter addition as the control) were used to explore the impacts of straw size on soil aggregation and the formation of aggregate-associated organic C. Although both straw sizes are unusual in the field, they are frequently used in laboratories to ensure that the straw can be thoroughly mixed with soil to achieve homogeneity. We chose wheat straw as a plant input source as wheat is

Table 2

The impacts of straw size and soil clay content on CO₂ respiration, the quality of dissolved organic matter (DOM), and enzyme activity during the 80-days incubation using the permutation test. *: P < 0.05; **: P < 0.01; ***: P < 0.001; ns: not significant.

Treatment effect	CO ₂ respiration		DOM quality		Enzyme activity	
	CO ₂ respiration rate	Cumulative CO ₂ emission	Dissolved organic carbon	SUVA ₂₅₄	β-glucosidase	cellobiohydrolase
Straw size (S)	***	*	***	ns	*	***
Clay content (C)	ns	ns	ns	ns	ns	ns
C × S	ns	ns	*	*	ns	ns

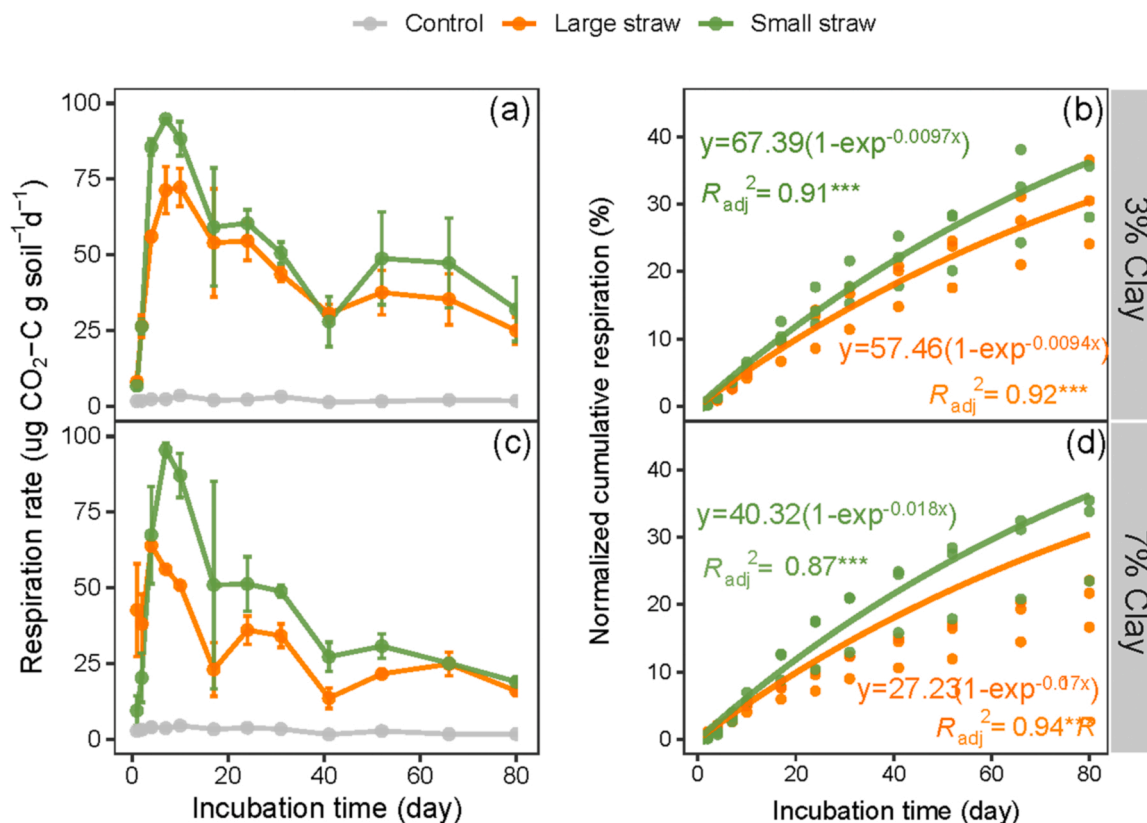


Fig. 1. The CO₂ respiration rate (a, c, µg CO₂-C g soil⁻¹ d⁻¹) and normalized cumulative respiration (b, d) after straw addition with time. The relationships between cumulative respiration and time are fitted by the exponential function of $*(1 - \exp(-k * x))$, where y represents cumulative respiration, and x represents incubation time. The data are mean ± standard error (n = 3). 3 % clay: 3 % clay, 42.7 % silt, and 54.3 % sand; 7 % clay: 7 % clay, 44.1 % silt, and 48.9 % sand. Large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw. The significance level: ns, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

the largest staple food crop worldwide. Wheat straw from a long-term fertilization experiment in Henan province, China, was oven-dried at 60 °C for 48 h, then shredded and sieved to the sizes of the 0.1–0.2 cm and < 0.25 mm.

To replicate natural sandy soils with low C sequestration capacity and test whether soil aggregates can form at extremely low clay content, we created artificial soil using two different clay contents with slight variations, specifically 3 % clay soil consisting of 3 % clay, 42.7 % silt, and 54.3 % sand and 7 % clay soil consisting of 7 % clay, 44.1 % silt, and 48.9 % sand, where the clay is clay-sized illite (<2 µm). The artificial soils consisted of quartz and illite minerals in different particle sizes, with comprehensive soil composition data in Table 1. The illite mineral (Hunyuan Fuhong Mineral Products Co., China) had the size of < 2 µm and < 53 µm and functioned as the active material that interacted with organic materials. In the artificial soils, we used quartz (shanghai Macklin Biochemical Co., Ltd, China) of three sizes, one silt size (<53 µm) and two sand sizes (53–250 µm and 250–2000 µm), to provide inert physical structural support (Bucka et al., 2021; Pronk et al., 2012). The mineralogy of illite and quartz was analyzed using X-ray diffraction.

To enhance the decomposition of wheat straw, we added microbial

inoculum extracted from arable topsoil to artificial soils, as described by Pronk (2012). Briefly, the extract was made by shaking soil and glass beads in a suspension at a soil-to-water ratio of 1:9 for two hours. Afterwards, the suspension was centrifuged at 1000 g for 12 min (step 1). Next, the resulting supernatant was moved to a new tube and centrifuged for 30 min at 3470 g. Lastly, water (ten times the weight of the soil from step 1) was added and thoroughly mixed to create the microbial inoculum. Each 100 g of artificial soil received 6 ml of microbial inoculum.

To achieve desired organic C concentration, we added 3 g of wheat straw (with organic C content of 36.80 %) to each 100 g of artificial soil, which contains a total of 1.03 g organic C. This is comparable to the amount of C added in the experiment conducted by Bucka et al. (2021). For each sample, we mixed 100 g artificial soils with 3 g dry weight of wheat straw, 6 ml microbial inoculant, and Hoagland's No.2 basal salt mixture (Sigma-Aldrich, Steinheim, Germany) with pH 5.5 diluted by 10 times (Bucka et al., 2021). Hoagland's solution was used to create a baseline soil solution, which has an ionic strength typical in natural soils and provides abundant nutrients for microorganisms. We added 12.99 ml and 15.25 ml diluted Hoagland's solution to each 100 g of 3 % clay

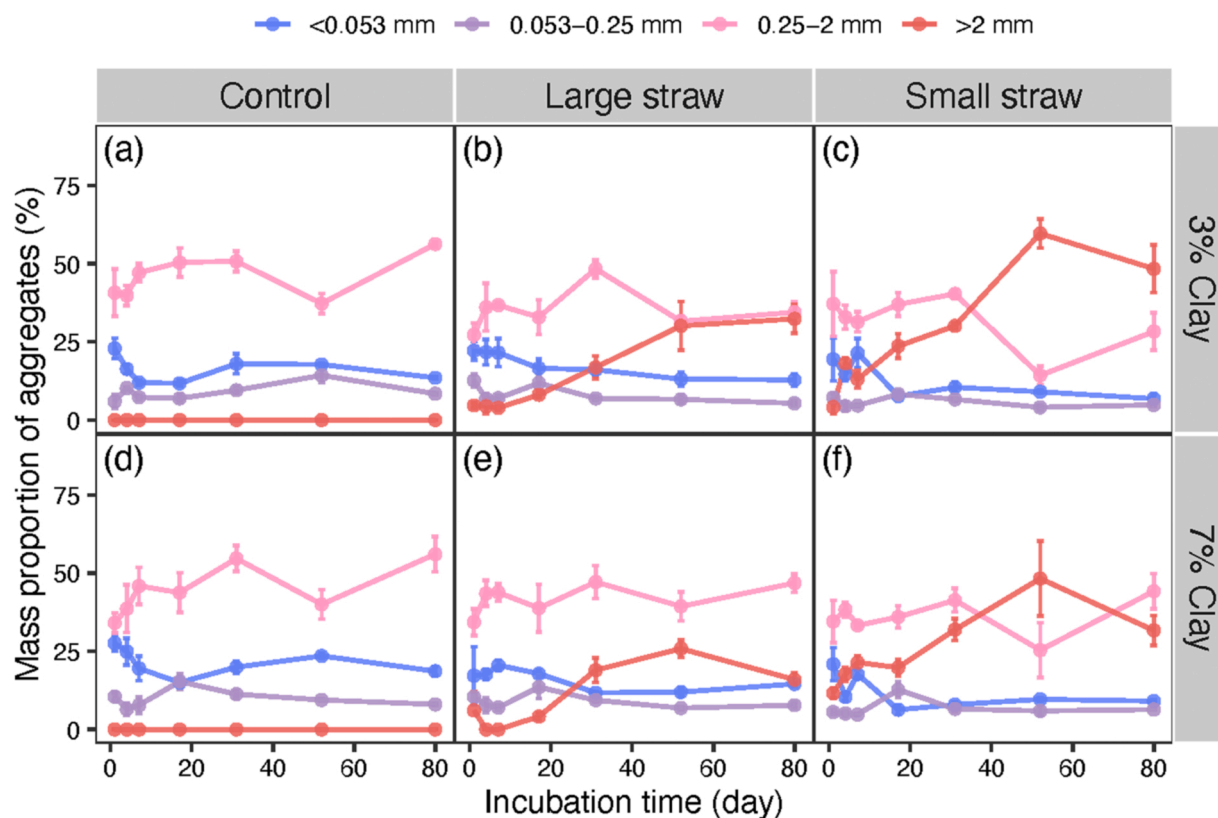


Fig. 2. Mass proportion of soil aggregates (%) after straw addition over time. The data are mean \pm standard error ($n = 3$). 3 % clay: 3 % clay, 42.7 % silt, and 54.3 % sand; 7 % clay: 7 % clay, 44.1 % silt, and 48.9 % sand. Control: no wheat straw addition; large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw; < 0.053 mm: small microaggregates; 0.053–0.25 mm: large microaggregates; 0.25–2 mm: small macroaggregates; > 2 mm: large macroaggregates.

soil and 7 % clay soil, respectively. The microbial inoculum and diluted Hoagland's solution were slowly added to the surface of pre-mixed solid components of the artificial soil using a dropper, allowing the liquid to seep down slowly without stirring. All samples were incubated under a constant dark condition at 25 °C with a water content set to 60 % water holding capacity for a duration of 80 days. The soil moisture content with 3 % clay content and 7 % clay content was 17.99 ± 0.59 % w/w and 20.25 ± 0.79 % w/w, with no significant difference.

2.2. CO₂ respiration rate assessment

The CO₂ respiration rate of the artificial soils with straw was routinely measured to determine the decomposition of added straw and SOC. Prior to each gas sampling, the jars were flushed with CO₂-free compressed air (21 % O₂ and 79 % N₂) for 1 min and sealed with airtight lids for 12 h. An open three-way valve allowed air to circulate both inside and outside the jars under aerobic conditions. We used a syringe to collect gas samples (50 ml) into the air collection bag and measured the respiration rate on the incubation days of 1, 2, 4, 7, 10, 17, 24, 31, 41, 52, 66, and 80. The CO₂ concentration was determined within 48 h after sampling by using Agilent 7890 A gas chromatography (Agilent Technologies, Santa Clara, CA, USA) equipped with a thermal conductivity detector. The CO₂ concentration of the samples was calculated using a six-point calibration curve with a standard gas having a known CO₂ concentration. The following equation was used to calculate CO₂ respiration rate ($\mu\text{g CO}_2\text{-C g soil}^{-1} \text{d}^{-1}$):

$$F = \rho \times \frac{V}{M} \times \frac{dc}{dt} \times \frac{273}{T}$$

Where ρ is the density of CO₂ (1.98 g L⁻¹) under standard conditions, V is the volume of the glass mason jar (L), M is the mass of incubated soil in each jar (g), dc/dt is the slope of the gas concentration curve variation

through time, and T is the incubation temperature (K). The “area-under-the-curve” method was used to determine cumulative CO₂ emission for each jar (Maucieri et al., 2017).

2.3. Aggregate fractionation

To examine changes in the mass proportion and organic C of soil aggregates over incubation time, we fractionated soils to aggregates by using the wet sieving method (Bucka et al., 2021). A sieve stack with mesh sizes of 2000 μm , 250 μm , and 53 μm was loaded with approximately 50 g of air-dried soil material, submerged in deionized water, and sieved for two minutes at a speed of 30 revolutions per minute with a soil aggregate analyzer (Xiangyu Weiye Instrument Beijing Co., Ltd., China). Residual soil above each sieve was flushed into aluminum boxes and oven-dried at 60 °C. As the artificial soils were created by including aggregate-sized sand grains (0.053–0.25 mm; 0.25–2 mm), it was necessary to perform sand correction on aggregate. To determine the sand content (0.053–0.25 mm and 0.25–2 mm), 5 g of dry soil aggregates of 0.053–0.25 mm and 0.25–2 mm were individually weighed. They were then mixed with 20 ml of 5 % (w/v) sodium hexametaphosphate to facilitate dispersion and passed through 0.053 mm and 0.25 mm sieves accordingly. The sieves were rinsed with distilled water and the final particles left on the sieve were sand particles within the aggregate of the corresponding size. Pure aggregate content was obtained by subtracting the mass of the sand particle from the mass of the aggregate before dispersion of the corresponding particle size. Subsequently, the mass proportion of the aggregate was calculated using the pure aggregate content (Elliott et al., 1991; Six et al., 2000; Tiemann et al., 2015). The organic C and N content of wheat straw, the artificial soil before and after incubation, and each soil aggregate before dispersion were analyzed by dry combustion with an elemental analyzer (Vario Macro C/N, Elementar, Germany). The organic C in pure aggregate was calculated using the

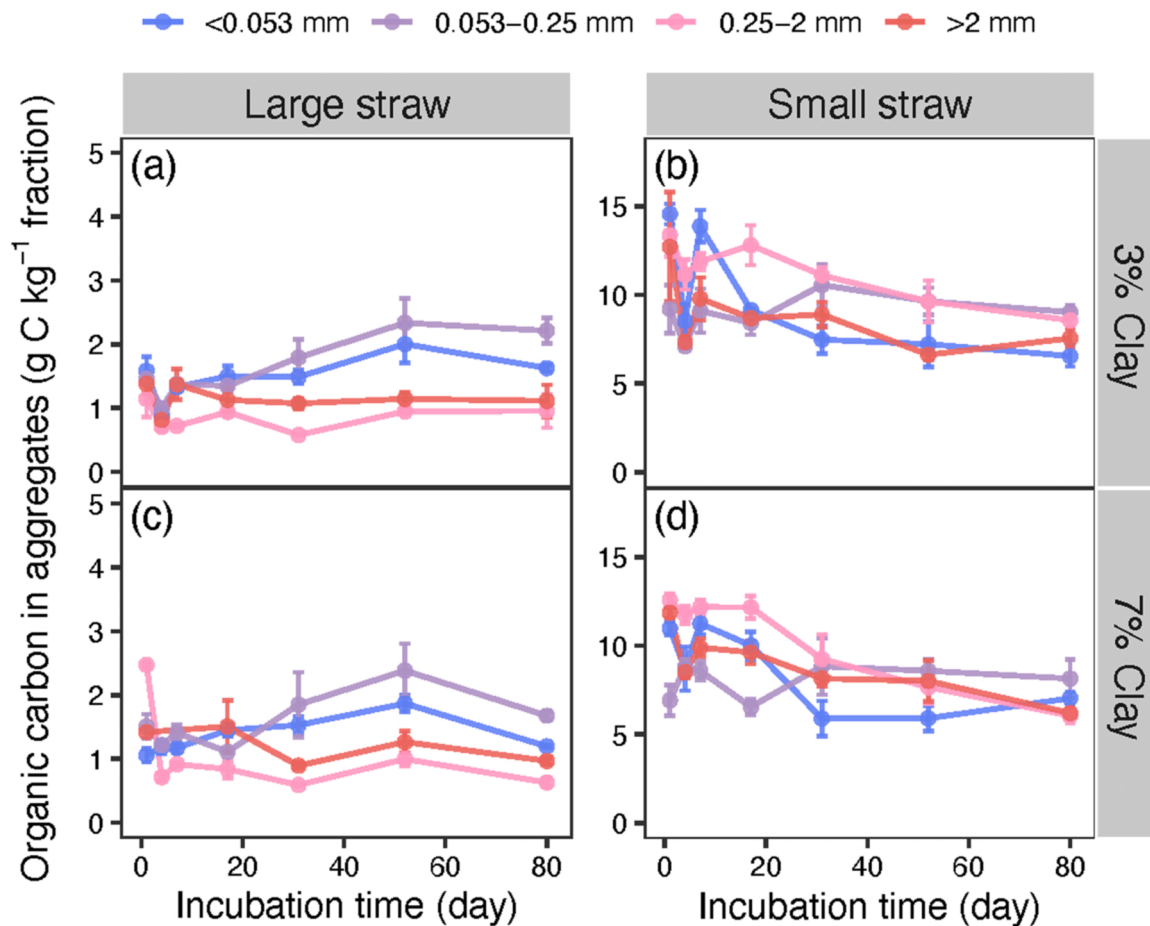


Fig. 3. Organic C concentration (g kg^{-1} fraction) of soil aggregates after straw addition over time. The data are mean \pm standard error ($n = 3$). 3 % clay: 3 % clay, 42.7 % silt, and 54.3 % sand; 7 % clay: 7 % clay, 44.1 % silt, and 48.9 % sand; Control: no wheat straw addition; large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw; <0.053 mm: small microaggregates; 0.053–0.25 mm: large microaggregates; 0.25–2 mm: small macroaggregates; > 2 mm: large macroaggregates.

following equation:

$$\text{OC in pure aggregate} \left(\text{g kg}^{-1} \right) = \frac{\text{OC in aggregate before dispersion} \left(\text{g kg}^{-1} \right) \times \text{mass of aggregate before dispersion} \left(\text{g} \right)}{\text{mass of aggregate before dispersion} \left(\text{g} \right) - \text{mass of sand} \left(\text{g} \right)}$$

2.4. Soil dissolved organic C and SUVA₂₅₄

To assess changes in organic matter composition of bulk soil after straw addition, we measured soil DOC and SUVA₂₅₄ of DOM. Briefly, 3 g of fresh soil was added to 30 ml of deionized water and shaken on a reciprocating shaker at 25 °C. The supernatant was obtained by centrifuging the solution at 4000 rpm for 40 min. The extracts (<0.45 μm) were then put into two 15 ml centrifuge tubes, with each tube filled with approximately 10 ml liquid. After acidification, a subsample was used to determine DOC using an Analytikjena multi-N/C 3100 TOC analyzer (Analytikjena, Germany). The absorption spectra of another extract aliquot were scanned from 190 to 800 nm at a 1 nm interval using a UV-visible spectrophotometer (UV-2550, Shimadzu, Japan), equipped

with a 150 W ozone-free xenon lamp. During the scanning, deionized water was used as a blank. The SUVA₂₅₄ was calculated by dividing the

UVA₂₅₄ (measured at a quartz cell path length of 1 cm) by the DOC concentration, as shown in the following equation (Abd Manan et al., 2020):

$$\text{SUVA}_{254} \left(\text{L mg}^{-1} \text{m}^{-1} \right) = \frac{\text{UVA} \left(\text{cm}^{-1} \right)}{\text{DOC} \left(\text{mg L}^{-1} \right)} \times 100 \left(\text{cm m}^{-1} \right)$$

A higher SUVA₂₅₄ value indicates greater aromaticity of DOM (Weishaar, 2003).

2.5. Enzymatic activities analyses

To examine the impacts of microbial activity on litter decomposition over the incubation period, we measured the potential activity of C-acquisition extracellular enzymes on the incubation days of 1, 7, 31, and 80 d. We measured the potential activities of two hydrolytic enzymes (β -1,4-glucosidase (BG) and cellobiohydrolase (CB)) because they are

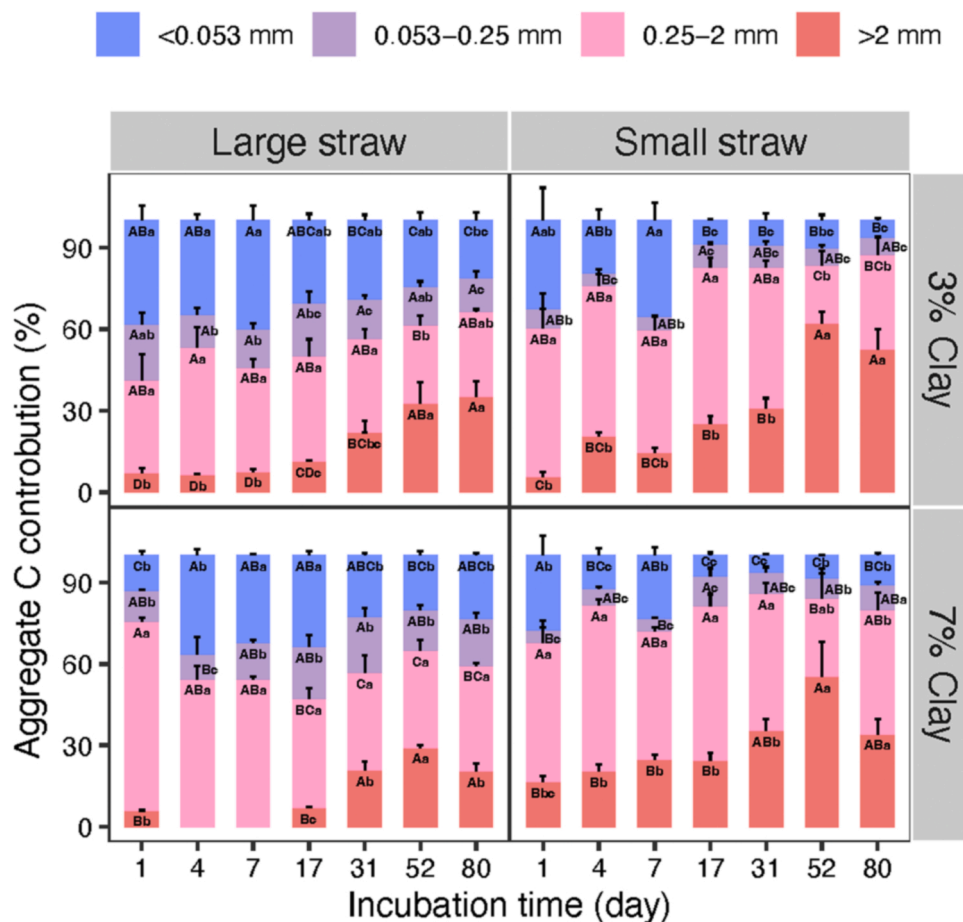


Fig. 4. The relative percentage of organic C in different aggregates after straw addition over time. Significant differences are indicated at the significance level of 0.05 by different uppercase letters for a given aggregate size and lowercase letters for a given incubation time. Error bars represent standard error for each aggregate. 3 % clay soil: 3 % clay, 42.7 % silt, and 54.3 % sand; 7 % clay soil: 7 % clay, 44.1 % silt, and 48.9 % sand; Control: no wheat straw addition; large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw. < 0.053 mm: small microaggregates; 0.053–0.25 mm: large microaggregates; 0.25–2 mm: small macroaggregates; > 2 mm: large macroaggregates.

related to the degradation of labile organic matter. Cellobiose is degraded by the BG enzyme into glucose, and the CB enzyme converts cellulose into disaccharides. We measured the potential activities of BG and CB by following the protocol established by Bell et al. (2013). Briefly, 150 ml sodium acetate buffer (50 mM, pH = 5.3) was combined uniformly with 1.5 g of fresh soil to create soil slurry sample. Soil slurries (200 μl) were moved to a black 96-well microplate with eight replicates for each soil sample. Then 50 μl of 200 μM BG or CB enzyme substrate, 4-Methylumbelliferone (MUB) was added and mixed with the soil slurries. For each soil slurry sample, we created a standard curve using MUB with a concentration gradient (0, 2.5, 5, 10, 25, 50, and 100 μM). The microplate was then incubated for 3 h in dark at 25 °C. Finally, the fluorescence of microplate samples was measured by a microplate reader (Thermo Scientific Fluoroskan Ascent FL, USA) with a 460 nm emission filter. The absolute enzyme activity of the soil was normalized to nmol g⁻¹ dry soil hr⁻¹.

2.6. Statistical analysis

To estimate the decomposition rate of straw, we fit the normalized cumulative CO₂-C emission with incubation time by using the first-order discrete-pool model (Liang et al., 2015).

$$R_{cum} = C_0(1 - e^{-kt})$$

Where R_{cum} is the normalized cumulative CO₂-C emission at time t (mg C g⁻¹ soil), C_0 is the initial soil C content (mg C g⁻¹ soil), and k is the input

litter decay rate (day⁻¹).

To quantify the amount of organic C in soil aggregates resulting from straw addition, the relative quantity of C stabilized in aggregate was calculated as follows:

$$\text{Relative quantity of C stabilized} = \frac{\text{C concentration of aggregate} \times \text{mass proportion of aggregate}}{\text{C input}} \times 100\%$$

We plotted the CO₂ respiration rate, the mass proportion of aggregates, organic C in soil aggregates, relative quantity of C stabilized in aggregates, DOC, and SUVA₂₅₄ against incubation time to display their temporal patterns. The data were presented as mean ± standard error (n = 3). Due to non-normal distribution of the data, we assessed the impacts of straw size and soil texture on the respiration rate and cumulative CO₂ emission using a permutation test, and significance threshold of α = 0.05 was established for every test. To explore potential factors driving changes in the relative quantity of C stabilized in aggregates, we performed a least square linear fitting between the relative quantity of C stabilized in aggregates and DOC, SUVA₂₅₄, and enzyme activity. Additionally, we examined the relationships between CO₂ respiration rate and extracellular enzyme activity by using the least squares linear fitting as well. All analyses were conducted with R (version 4.0.1, R Development Core Team 2020) by using 'ggplot2', 'stats', and 'agricolae' packages for nonlinear, linear regression models, and time patterns.

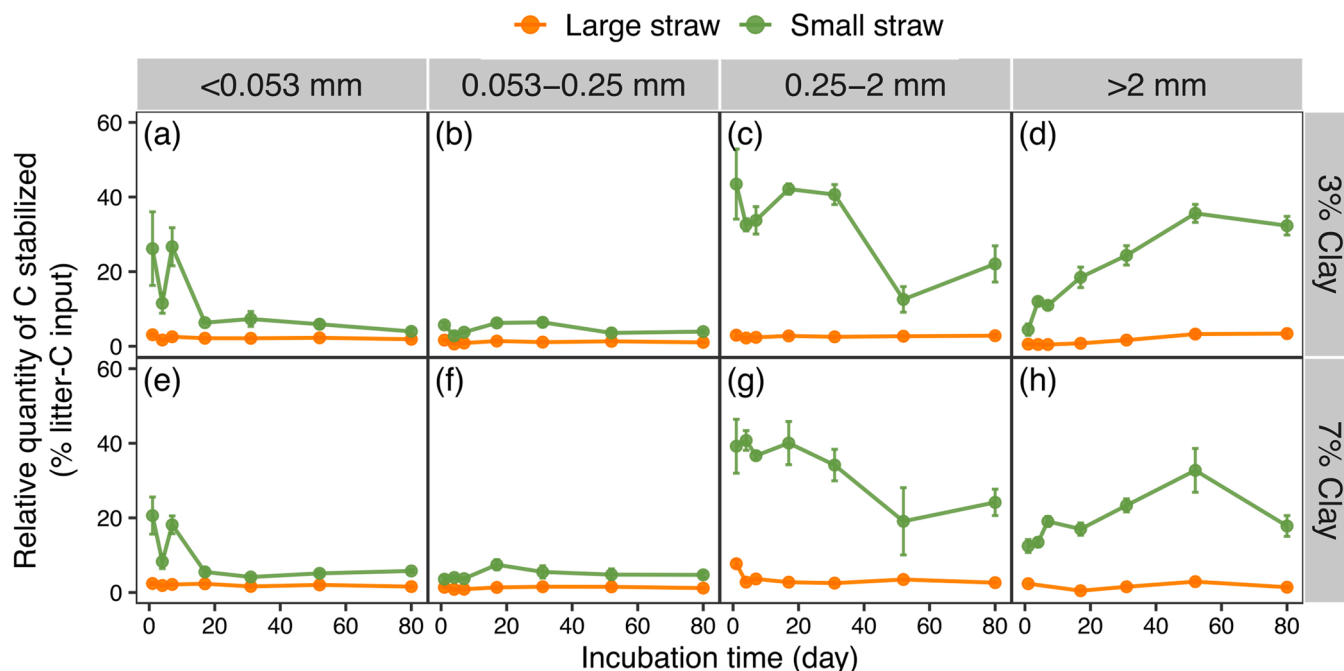


Fig. 5. The relative quantity of C stabilized in soil aggregates (%) after straw addition over time. The data are mean \pm standard error ($n = 3$). 3 % clay soil: 3 % clay, 42.7 % silt, and 54.3 % sand; 7 % clay soil: 7 % clay, 44.1 % silt, and 48.9 % sand; Control: no wheat straw addition; large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw. <0.053 mm: small microaggregates; 0.053–0.25 mm: large microaggregates; 0.25–2 mm: small macroaggregates; > 2 mm: large macroaggregates.

3. Results

3.1. Respiration rate and relative quantity of C stabilized in aggregates over time

Only straw size, not soil texture, had a significant effect on CO_2 respiration rate and cumulative CO_2 emissions (Table 2). The CO_2 respiration rate of the 3 %-clay soil was higher than that of the 7 %-clay soil, regardless of straw size. Adding small straw led to a higher CO_2 respiration rate and normalized cumulative respiration compared to the addition of large straw, regardless of soil clay content (Fig. 1).

The mass proportion of large macroaggregate (>2 mm) increased over time, while the mass proportion of small macroaggregate (0.25–2 mm) decreased. These opposite trends were most pronounced in the soil with 3 % clay content (Fig. 2b, c). Furthermore, the C concentrations of soil aggregates in the treatment with small straw addition decreased with incubation time (Fig. 3b, d). In contrast, the C concentrations of all soil aggregates in the treatment with large straw addition (0.58–2.35 g kg^{-1}) were lower than those in the treatment of small straw addition (6.56–13.31 g kg^{-1}) and did not exhibit significant changes over time (Fig. 3a, c). Straw size significantly affected the mass proportion and organic C of aggregates of different sizes, but soil texture did not show any notable effect (Table S1).

The relative contribution of organic C in large macroaggregate (>2 mm) to total SOC increased, while that of small microaggregate (<0.053 mm) decreased over incubation time (Fig. 4). Both soil texture and straw size significantly affected the contribution of C to total SOC in small microaggregate (<0.053 mm) and small macroaggregate (0.25–2 mm) to total SOC (Table S1). The relative quantity of C stabilized in all the soil aggregates was higher in the treatment with small straw addition than in the treatment with large straw addition, particularly in the early stage of incubation. This difference was most notable for the large (>2 mm) and small (0.25–2 mm) macroaggregate and small microaggregate (<0.053 mm) (Fig. 5). However, the relative quantity of C stabilized in large microaggregate (0.053–0.25 mm) remained unchanged throughout the incubation period in all treatments

(Fig. 5b, f). In addition, only straw size had a significant impact on the relative quantity of C stabilized in soil aggregates of various sizes (Table S1).

3.2. Change in quantity and quality of DOM and enzyme activities over incubation time

The DOC concentration was significantly higher in the treatment with small straw addition than in the treatment with large straw addition, particularly during the first 20 days of incubation (Fig. 6a, c). Additionally, SUVA_{254} exhibited an increasing trend with time but showed no significant difference between the treatments with large and small straw addition (Fig. 6b, d).

The potential activities of two hydrolytic enzymes (BG and CB) were significantly higher in the treatment with small straw addition than those in the treatment with large straw addition (Fig. S1). Straw size had a significant effect on enzyme activity, whereas soil texture did not show significant impact (Table 2). Furthermore, the activities of BG and CB enzymes exhibited temporal variability. Specifically, these activities were very low at the onset of incubation, reached the maximum on day 7, and subsequently decreased overtime (Fig. S1).

The relative quantity of C stabilized in different-sized soil aggregates exhibited varying relations with DOC. Specifically, in the treatment with small straw addition, the relative quantity of C stabilized in small microaggregate (<0.053 mm) was positively correlated with DOC, whereas in large macroaggregate (>2 mm) it was negatively correlated (Fig. 7a, d). Furthermore, the relative quantity of C stabilized in small microaggregate (<0.053 mm) decreased with SUVA_{254} after straw addition in both the small and large straw addition treatments (Fig. 7e). In contrast, the relative quantity of C stabilized in large macroaggregate (>2 mm) increased with SUVA_{254} after straw addition, also regardless of straw size (Fig. 7e–h). Meanwhile, there was a significant positive correlation between BG activity and the relative quantity of C stabilized in small microaggregate (<0.053 mm) only in the treatment of small straw addition (Fig. 8). Additionally, in soils with 3 % clay content after straw addition, the CO_2 respiration rate was positively correlated with the

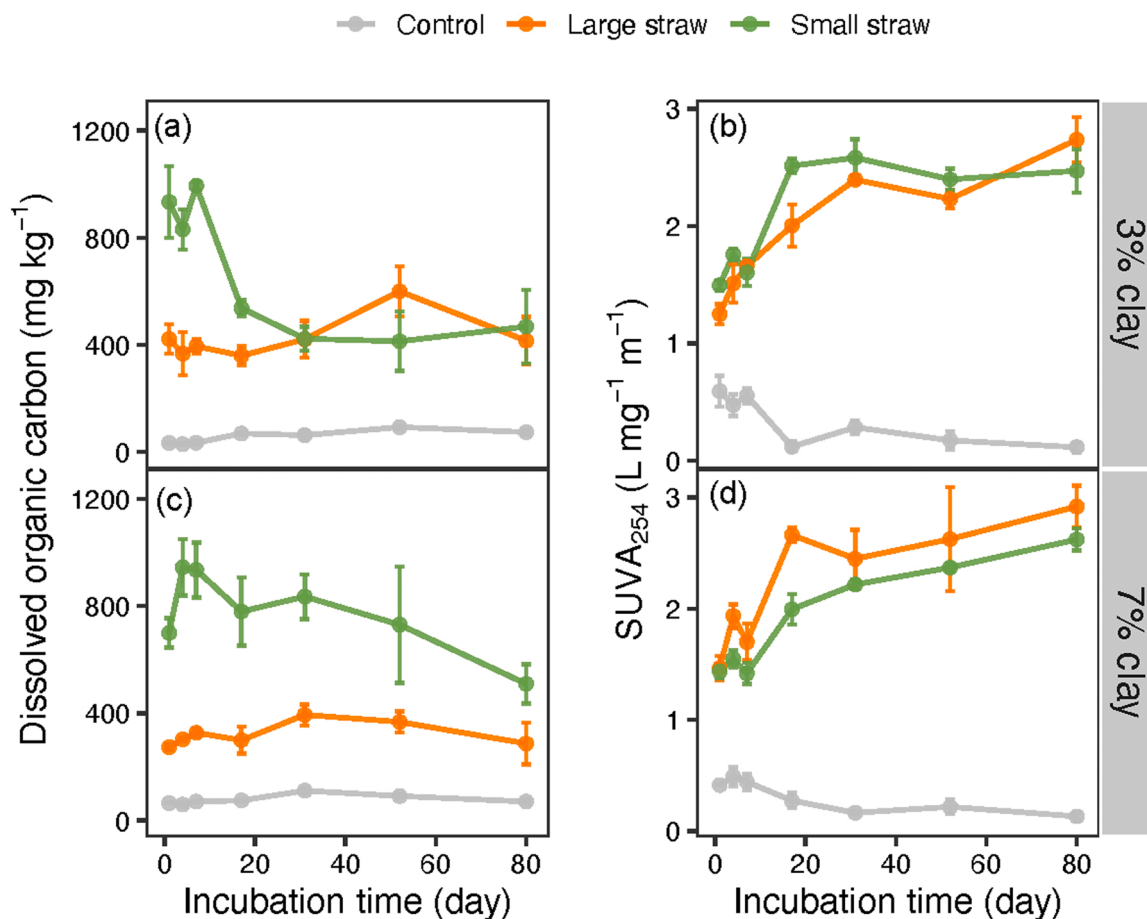


Fig. 6. Dissolved organic carbon (mg kg^{-1}) and specific ultraviolet absorbance at 254 nm (SUVA_{254} , $\text{L mg}^{-1} \text{m}^{-1}$) of bulk soil over time. The data are mean \pm standard error ($n = 3$). 3 % clay: 3 % clay, 42.7 % silt, and 54.3 % sand; 7 % clay: 7 % clay, 44.1 % silt, and 48.9 % sand; Control: no wheat straw addition; large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw.

activities of BG and CB, regardless of straw size, whereas in 7 % clay soil only a significant positive relationship of BG with respiration rate was found in small straw. (Fig. 9).

4. Discussion

4.1. Effect of straw size and clay content on decomposition and relative quantity of C stabilized in aggregates

Our results indicated that small straw decomposed faster than large straw, as demonstrated by higher respiration rate and greater CO_2 loss in the treatment with small straw addition. This finding is consistent with the results of other studies. Zhang et al. (2020) reported that the addition of small maize straw (<0.15 mm) resulted in high cumulative CO_2 respiration than the addition of large maize straw (<0.25 mm). Similarly, Bucka et al. (2021) found that small-sized hay litter (<0.63 mm) released more CO_2 than large-sized hay litter (0.63–2 mm). It must be due to significantly higher enzyme activity found in the treatments with small straw addition than with large straw addition (Fig. S1). More microorganisms can colonize on small straws than on large straws due to larger specific surface area, and small straws are more likely to release nutrients that allow microorganisms to produce enzymes to decompose straw, leading to significantly higher enzyme activity (Rinkes et al., 2013). The positive linear relationship between enzyme activity and respiration rate we found (Fig. 8) indicates that enzyme activity governed straw decomposition. However, we did not find similar relationships between respiration rate and enzyme activity in the 7 % clay soil. It is probably that microbes have limited contact with straw in the soil

with higher clay content.

So far, no studies have explored the impacts of straw size on the relative quantity of C stabilized in soil aggregates, despite previous research showing that belowground inputs result in higher MAOC formation efficiency than aboveground inputs (Villarino et al., 2021). Wei et al. (2022) demonstrated that the efficiency of SOC formation after the addition of *Stipa kralovii* varied widely in an 80-day field soil incubation in different soil fractions. Furthermore, we discovered that the relative quantity of C stabilized in the small straw addition treatment (0.41–21.06 %) was higher than in the large straw addition treatment (0.22–3.98 %). This finding could be attributed to small straw being more accessible to microorganisms than large straw, which can stimulate microorganisms to produce not only more extracellular enzymes for straw decomposition but also more DOC and microbial biomass. These substances are precursors to SOC formation (Cotrufo et al., 2013; Liang et al., 2017). According to Wang et al. (2022), high enzyme activity leads to higher SOC as the anabolic process contributes to microbial biomass, necromass, and extracellular polymers.

There are mixed results regarding the impacts of soil clay content on litter decomposition and aggregate formation. Typically, litter decomposition is slower (Angst et al., 2021) and aggregate formation is more pronounced in soils with higher clay content than those with lower clay content (Schweizer et al., 2019). However, our results revealed that soil clay content had no significant effect on CO_2 respiration rate, mass proportion of aggregate, and organic C in aggregate. This could be due to the narrow range of soil clay content tested (3 % vs. 7 %), which prevented any impact on aggregate formation. De Gryze et al. (2005) also found no significant impact of clay content on aggregate formation

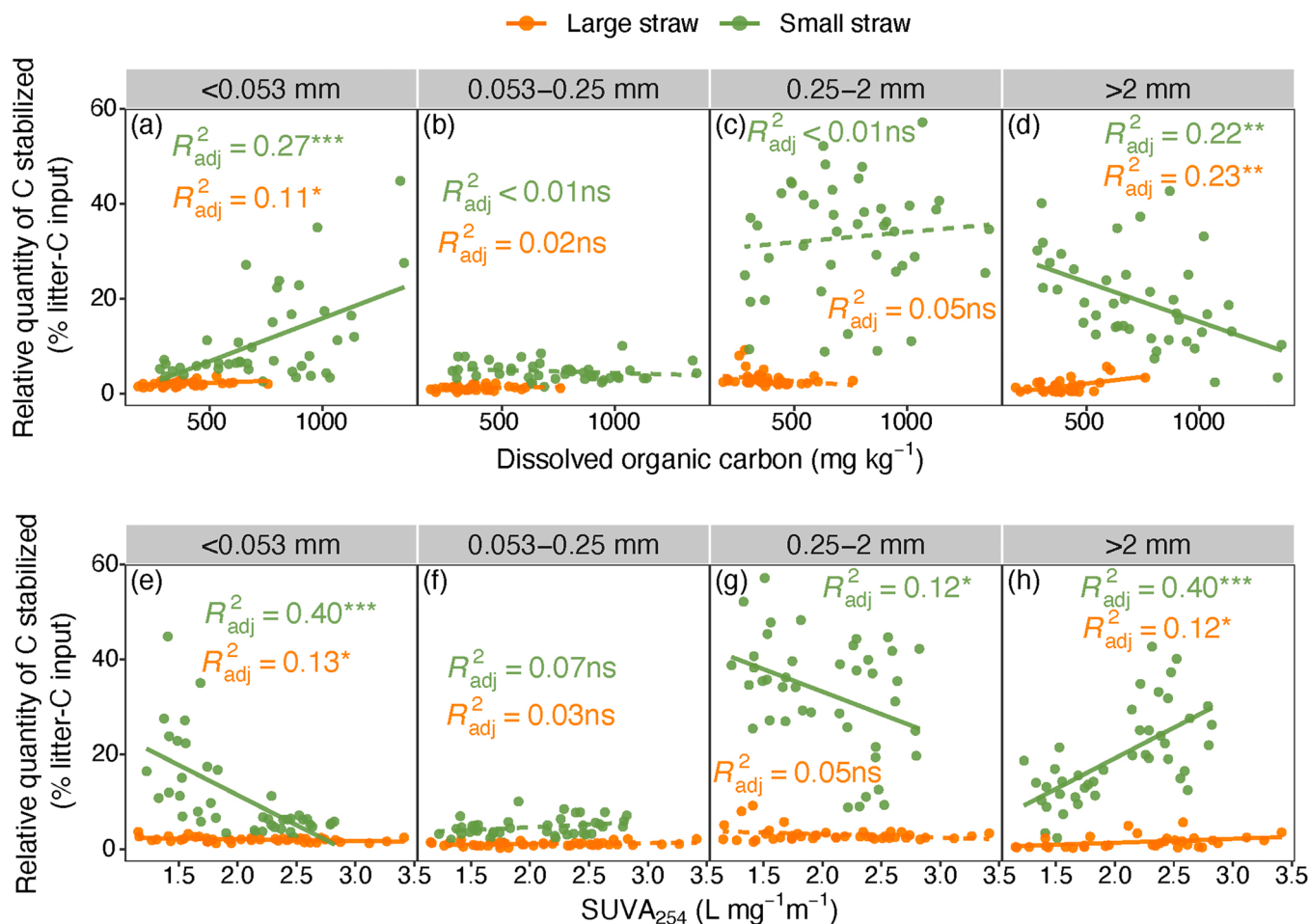


Fig. 7. The relationships between the relative quantity of C stabilized in soil aggregates and dissolved organic C and SUVA₂₅₄. Large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw. <0.053 mm: small microaggregates; 0.053–0.25 mm: large microaggregates; 0.25–2 mm: small macroaggregates; >2 mm: large macroaggregates. The significance level: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

among three soil types (sandy loam, silt loam, and silty clay loam). Therefore, the effects of soil clay content on litter decomposition and soil aggregation are only noticeable when the variation of clay content exceeds a certain threshold. This presents an opportunity for further exploration.

4.2. Dynamics of aggregation and relative quantity of C stabilized in soil aggregates

Our results revealed that the relative quantity of C stabilized in small (0.25–2 mm) macroaggregates decreased with time, while that of large (>2 mm) macroaggregates increased. These opposing temporal patterns of two macroaggregates (0.25–2 mm and >2 mm) indicate interconversion and that large aggregates were primarily transformed from small aggregates in the late stage of incubation. Similar interconversion patterns were observed in natural soils incubated for 21 days (Blaud et al., 2012) and 76 days (Liu et al., 2020), as well as artificial soils incubated for 30 days (Bucka et al., 2021). It is possible that microaggregates in artificial soils are formed by the physical or electrostatic adsorption of a portion of small litter onto clay minerals, which is then combined into large aggregates by polysaccharide binding agents and fungal hyphae produced during microbial decomposition. This process is consistent with the concept of aggregate hierarchy (Oades, 1984; Tisdall and Oades, 1982). Meanwhile, we found that the proportion of 0.053–0.25 mm aggregate remained unchanged during the incubation period. This could be that the original material used in our study was pure quartz sand, which is an inert material.

Our findings demonstrate that the relative quantity of C stabilized in soil aggregates reached maximum at different times. To our knowledge, no study has focused on how the relative quantity of C stabilized in soil aggregates varies with time. Small microaggregate (<0.053 mm) and small macroaggregate (0.25–2 mm) exhibited high relative quantity of C stabilized during the initial 30-day incubation period, whereas large macroaggregate (>2 mm) reached their maximum in the late stage of incubation (52 days) (Fig. 5). Additionally, the temporal variations of the relative quantity of C stabilized in aggregates we found can be attributed to their mass proportion, as the relative quantity of C stabilized in soil aggregates was determined by their C concentration and mass proportion. Our findings showed that the former (organic C concentration) of different-sized aggregates was similar over incubation time. However, the latter (mass proportion) varied with time, with the mass proportions of the small macroaggregate (0.25–2 mm) decreasing and the large macroaggregate (>2 mm) increased (Fig. 3). Therefore, our study demonstrates that the relative quantity of C stabilized from litter to soil is significantly influenced more by the formation and breakdown of aggregates rather than the C concentrations of aggregates.

4.3. The pathway of the formation of aggregate associated organic C

Our results indicate that the labile component of organic matter governed the formation of small micro-aggregate associated C (<0.053 mm) in the early stage of incubation, whereas the structural component of organic matter regulated the formation of large macro-aggregate (>2 mm) associated C in the late stage. Our findings support

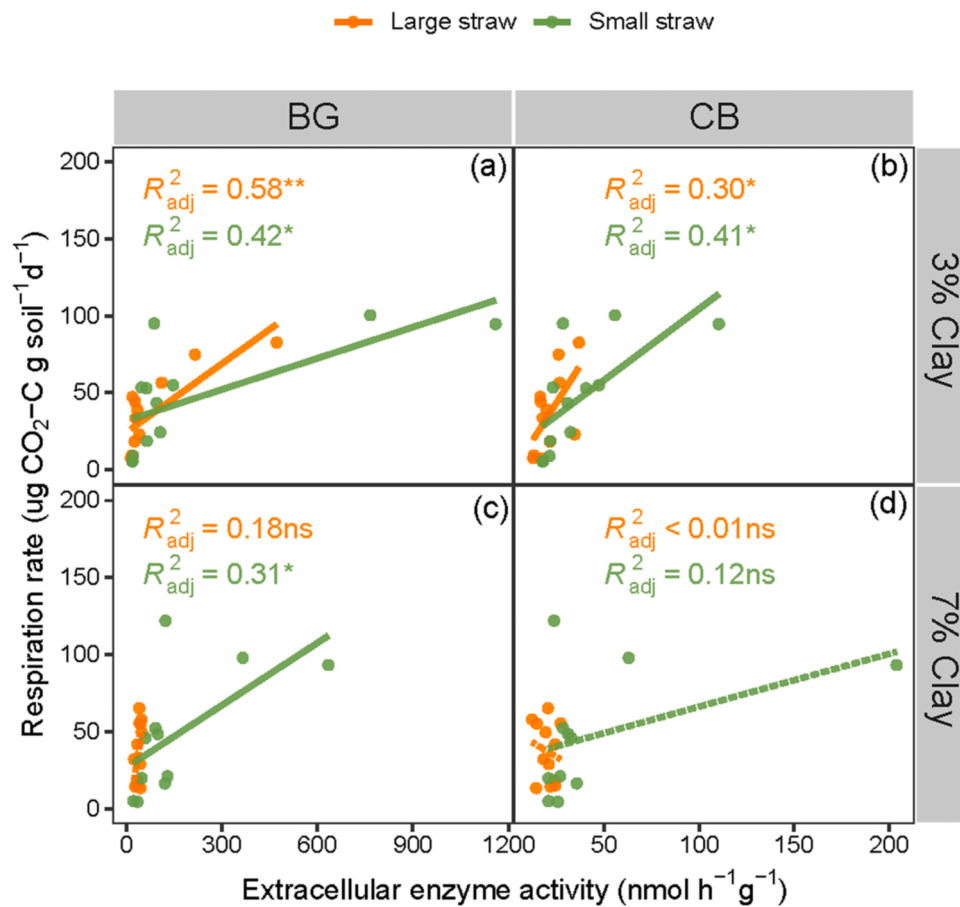


Fig. 8. The relationships between extracellular enzyme activity ($\text{nmol h}^{-1} \text{g}^{-1}$) and CO_2 respiration rate ($\mu\text{g CO}_2\text{-C g soil}^{-1} \text{d}^{-1}$) after straw addition over time. 3 % clay soil: 3 % clay, 42.7 % silt, and 54.3 % sand; 7 % clay soil: 7 % clay, 44.1 % silt, and 48.9 % sand. Control: no wheat straw addition; large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw. BG: β -glucosidase; CB: cellobiohydrolase. The significance level: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

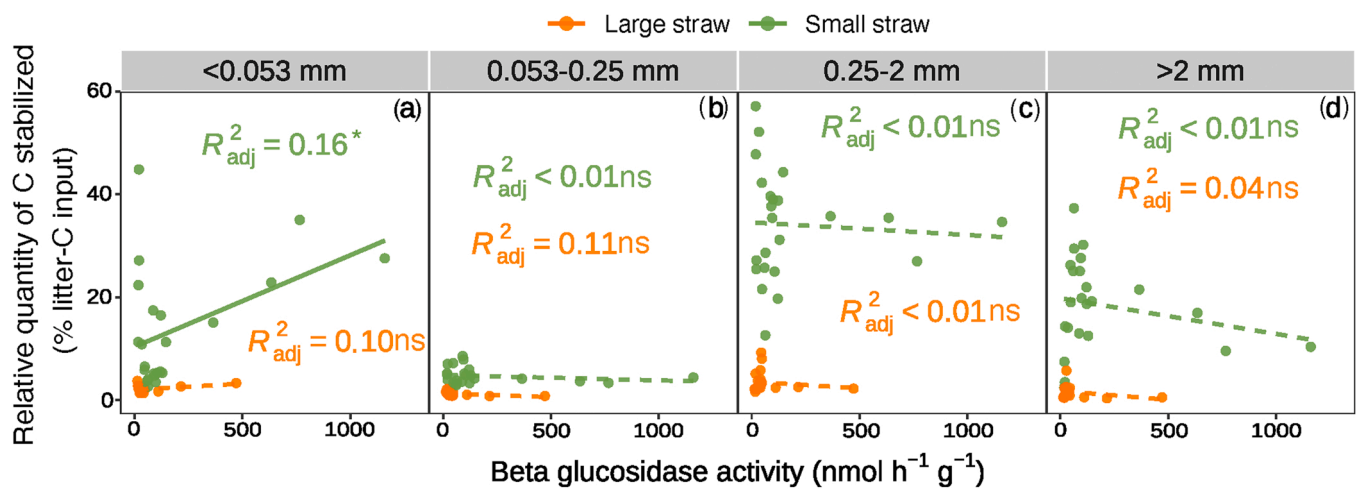


Fig. 9. The relationships between beta glucosidase activity ($\text{nmol h}^{-1} \text{g}^{-1}$) and the relative quantity of C stabilized in soil aggregates (%) after straw addition overtime. Large straw: 1–2 mm of wheat straw; small straw: <0.25 mm of wheat straw. < 0.053 mm: small microaggregates; 0.053–0.25 mm: large microaggregates; 0.25–2 mm: small macroaggregates; > 2 mm: large macroaggregates. The significance level: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

the DOC-microbial pathway for SOC formation proposed by Cotrufo et al. (2015), which suggests that non-structural soluble compounds (e. g., DOC) were incorporated into mineral to form microaggregate during the early stage of litter decomposition. Recent study found that labile leaf litter contributed more to the formation of mineral associated organic matter, while recalcitrant root litter contributes more to

particulate soil fractions (Fulton-Smith and Cotrufo, 2019). According to the density fractionation result, Haddix et al. (2016) discovered that all organic C in organo-mineral complexes came from metabolic litter component, while the structural litter component was only present in the light fraction. The results of our study demonstrate strong empirical evidence for the positive relationship between DOC and the relative

quantity of C stabilized in small microaggregate (<0.053 mm) (Fig. 7a). Our work indicates that the illite mineral in the clay-sized (<2 μm) fraction of the artificial soil was responsible for DOC adsorption and the subsequent formation of stable soil organic C. We found the relative quantity of C stabilized in small microaggregate (<0.053 mm) increased linearly with DOC (Fig. 7a). Our results offer evidence and support that DOC is the main determinant for the formation of stable soil organic C (e.g., microaggregate associated C), especially in the early stage after litter addition. These findings underscore the importance of quantifying DOC dynamics to accurately predict SOC formation. Additionally, our results suggest that the structural component of litter was preferentially stabilized by macroaggregate in the late stage of incubation. This was supported by the positive correlation between the relative quantity of C stabilized in large macroaggregate (>2 mm) and SUVA₂₅₄, an indicator of the abundance of aromatic molecules in DOC (Li et al., 2022). Li et al. (2022) found that increased SOC content across croplands in northwest China was mainly due to the accumulation of recalcitrant organic C fraction (e.g. aryl and phenolic C functional groups). Therefore, our findings highlight distinct pathways for the formation of soil microaggregate and macroaggregate, wherein the quantity and quality of DOM mediated by enzyme activity play a central role in regulating the relative quantity of C stabilized in various soil aggregates.

5. Conclusions

This laboratory experiment with different-sized straw addition reveals that straw size significantly affects soil aggregate formation and the relative quantity of C stabilized in aggregates. Our findings indicate that compared to large straw (2 mm), adding small straw (<0.25 mm) promotes litter decomposition and increases the relative quantity of C stabilized in all soil aggregates over time, which is aligned with our first hypothesis. Meanwhile, our research indicates that the narrow difference of soil clay content (3 % vs. 7 %) did not significantly affect aggregation and the relative quantity of C stabilized in various aggregates of sandy soil. Moreover, the quantity and quality of DOC significantly mediated by hydrolytic enzyme activity regulated the relative quantity of C stabilized in varying aggregates after the addition of small straw. Particularly, the quantity of DOC influenced the relative quantity of C stabilized in microaggregate (<0.053 mm), while the aromaticity of DOM (SUVA₂₅₄) affected the relative quantity of C stabilized in macroaggregate (>2 mm). This study suggests that adding small straw is an effective way to improve soil C sequestration and soil structure, and that both the quantity and quality of DOM governs the relative quantity of C stabilized in soil aggregate associated C.

CRedit authorship contribution statement

Wenting Feng: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Yugang Wang:** Writing – review & editing, Visualization, Resources, Funding acquisition, Conceptualization. **Gilles Colinet:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Xiaofang Ji:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiang Jiang:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2024.106022.

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