#### **ORIGINAL PAPER**



# **Soil Carbon Mineralization and Aggregate Distribution in Various Tillage Practices of Rice–Wheat Cropping System: A Field and Laboratory Study**

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### **Abstract**

Diferent tillage and residue management practices can strongly impact soil structure stability and soil organic carbon (SOC) sequestration. However, the detailed information about the aggregate stability, SOC protection, and mineralization within aggregates are still lacking. Using aggregate fractionation with laboratory incubation, we investigated aggregate-associated SOC, soil structural stability, and SOC mineralization in rice–wheat rotation under diferent tillage treatments: CT0 (puddled rice, conventional wheat − residue); CTR (puddled rice, conventional wheat + residue); NT0 (direct rice seeding, zero-tilled wheat − residue); and NTR (direct rice seeding, zero-tilled wheat + residue). NTR signifcantly enhanced the large macro-aggregate fraction (> 2 mm) at the 0–45 cm soil layer and macro-aggregate-associated SOC at the 0–15 cm soil layer. However, CTR enhanced the macro-aggregate-associated SOC at the 15–30 cm layer. Notably, the mean weight diameter (~8%) and geometric mean diameter (~24%) were higher under NTR than those under other treatments, and the effect was more pronounced in 30–45 cm layer. The highest average cumulative carbon mineralization  $C_m$  (~9%) was observed in macro-aggregates ( $> 2$  mm) than micro-aggregates ( $< 2$  mm). With regard to tillage systems, the  $C_m$  was higher under NTR compared to other treatments. However,  $C_m$  at the 15–30 cm layer was higher (~22%) under CTR than that in other treatments. Notably, a positive relationship was found between total carbon input and soil aggregation. Specifcally, carbon input of NT0, NTR, and CTR increased  $> 2$  mm aggregates at 0–15 cm, while carbon input of CTR increased  $> 2$  mm at 15–30 cm soil depth. Overall, no tillage with residue return (NTR) could enhance the soil macro-aggregation and associated SOC accumulation by decreasing SOC mineralization in rice–wheat double cropping system.

**Keywords** Carbon mineralization · Aggregate distribution · Tillage · Residue returning · Rice–wheat rotation

# **1 Introduction**

By the end of twenty-frst century, the average temperature rise by 3.7 °C due to unprecedented rise in the atmospheric carbon dioxide  $(CO<sub>2</sub>)$  has accelerated the global warming

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and climate change (IPCC [2013](#page-13-0)). Globally, crop production is a signifcant source of greenhouse gasses (GHGs) emission (Carlson et al. [2016](#page-13-1)) but also has potential to sequester soil organic carbon (SOC) and mitigate change in climate  $(Lal 2004; Lal 2016)$  $(Lal 2004; Lal 2016)$  $(Lal 2004; Lal 2016)$  $(Lal 2004; Lal 2016)$ . Therefore, offsetting atmospheric

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carbon (C) in terrestrial ecosystem and mitigating GHGs emissions are among key solutions to addressing climate change (IPCC [2018;](#page-13-2) Yang et al. [2023](#page-15-0)). Soil aggregates physically protect SOC and almost 90% of its sequestration occurs in soil aggregates (Andruschkewitsch et al. [2014](#page-13-3); Somasundaram et al. [2017](#page-15-1)). Each aggregate fraction has capacity to encapsulate the SOC with varying magnitude of physical protection against microbial degradation and moderated by diferent farmland management techniques (e.g., tillage and residue returning) (Spohn and Giani [2010](#page-15-2); Gelaw et al. [2015\)](#page-13-4). Furthermore, the buildup and turnover of SOC in farmlands based on the balance between carbon inputs primarily sources from crop residue retention (above-ground, root biomass, and rhizodeposition) and outputs via SOC mineralization by microbes (Malhi et al. [2011](#page-14-2); Xie et al. [2017](#page-15-3)). Thus, it is pertinent to evaluate the distribution, stabilization, and SOC mineralization in diferent proportions of soil aggregates to understand the mechanism of aggregate formation and SOC protection within aggregate classes.

Soil conservation practices (no tillage and residue retention) afect nutrient distribution and efectively transform SOC and aggregate stability (Zhao et al. [2015](#page-15-4); Somasundaram et al. [2018](#page-14-3)). Conventional tillage (CT) has been reported a major cause to physically disrupt large aggregates, making fner aggregates, thus destroying SOC binding in carbon-rich macro-aggregates and promoting SOC microbial degradation (Álvaro-Fuentes et al. [2008a,](#page-13-5) [2008b\)](#page-13-6). However, conservation tillage (e.g., no till, NT) results in less soil aggregate disruption at surface soil layer (0–10 cm depth), compared to CT. Furthermore, NT system promotes aggregate stability and carbon-rich formation of macro-aggregates (Sarker et al. [2018a](#page-14-4), [2018b](#page-14-5)). In general, macro-aggregates are more stable and richer in SOC compared with micro-aggregates. Accordingly, more macro-aggregates indicated higher soil structure stability and mean weight diameter (MWD) values and less mineralization (Wang et al. [2019\)](#page-15-5). Soil aggregate distributions are determined by the process of wet sieving to evaluate the infuence of diferent tillage and farmland management practices (Bottinelli et al. [2017](#page-13-7)). The process of wet sieving can dissolve organic matter within an aggregate fraction, transfer microbial populations from macro-aggregates  $(> 2 \text{ mm and } 2 - 0.25 \text{ mm})$  to micro-aggregates  $(0.25 - 0.053)$  $mm$  and  $< 0.053$  mm), and considerably disturb the microbial habitat (Gunina and Kuzyakov [2014](#page-13-8)), thereby disturbing mineralization mechanism (Kan et al. [2020a\)](#page-14-6). However, dry sieving could be useful in low SOC concentration soil to better analyze the carbon cycling mechanism. Thus, it is necessary to understand the process and mechanism that strengthen scientifc understanding regarding SOC mineralization, aggregate stability, and associated C contents under diferent tillage practices by adopting dry sieving technique.

Prior laboratory incubation studies have provided some useful insights of SOC mineralization under diferent tillage systems, even though with diferent results (Jacobs et al. [2010](#page-13-9)). For example, CT enhanced SOC (26–114%) mineralization in diferent aggregate fractions compared with NT in a Luvisol, however, had no efect in a Vertisol (Sarker et al. [2018b](#page-14-5)). In contrast, Fernández et al. ([2010\)](#page-13-10) reported that C mineralization was higher under NT compared with CT in all aggregate size classes for some soils in Argentina. Moreover, the magnitude of mineralization in aggregate fractions lacks a consensus. Typically, in comparison with micro-aggregates  $(0.25-0.053 \text{ mm}$  and  $< 0.053 \text{ mm}$ ), macro-aggregates ( $> 2$  mm and 2–0.25 mm) contain fresh labile C with greater microbial turnover and cumulative C mineralization rate (Fernández et al. [2010;](#page-13-10) Cai et al. [2016](#page-13-11)). In contrast, Rabbi et al. [\(2014\)](#page-14-7) stated that there were no differences in C mineralization, when comparing macro-aggregates with micro-aggregates. Another recent study indicated that higher magnitude of cumulative C mineralization was in micro-aggregates compared with macro-aggregates (Xie et al. [2017\)](#page-15-3). Such contradictory results are partly due to fact that SOC is heterogeneous in nature and partly because the grading or sieving size of aggregates was not homogenous in all conditions. Thus, in-depth understanding requires how aggregates protect, stabilize, and store SOC under particular soil type and climatic conditions in diferent macro- and micro-aggregate fractions to identify climate-smart tillage practice and to strengthen the soil aggregation hierarchy and SOC stability at regional scale.

Rice–wheat cropping system (RWCS) is the principal production system in the Indo-Gangetic Plains (IGP) and practiced on ~13.5 Mha area (Ladha et al. [2003](#page-14-8)). Historically, CT is usually followed by burning or removal of crop residues. However, long-term CT operation in the IGP continuously decreases soil structure and SOC stability due to residue burning (Mamta et al. [2022](#page-14-9)). Therefore, recommended soil conservation practices could stabilize soil aggregates and associated C contents and improve agricultural sustainability (Bhattacharyya et al. [2012\)](#page-13-12). However, to date, research information is lacking on how diferent tillage practices directly infuence the aggregate size distribution and associated C content in diferent sub-soil layers. Moreover, the mechanisms of soil structural stability, SOC protection, and mineralization in aggregates under various tillage systems for specifc RWCS in the IGP have not been systematically addressed. Furthermore, the relationships between C input and soil aggregation under various tillage systems are still not well understood.

Hence, this study is aimed to assess the soil aggregate size distribution (using dry sieving technique), associated C contents, soil structural stability, and mineralization in diferent aggregate fractions and soil layers under diferent tillage systems and residue management practices. It is based on the hypothesis that no till with residue retention would (i) increase the macro-aggregates fraction, (ii) promote SOC contents in macro-aggregates, and (iii) decline cumulative C mineralization capacity because of C encapsulation in the macro-aggregates. Consequently, the specifc aims of the study were to (i) evaluate the soil aggregate size distribution, structural stability, and associated SOC concentration under diferent tillage systems, (ii) investigate the cumulative C mineralization in various tillage systems, and (iii) identify the relationships between total C input and soil aggregation under diferent tillage and soil depths.

# **2 Materials and Methods**

#### **2.1 Field Experiment**

The feld experiment was initiated in June 2016 at Sukheki Farm, Hafzabad (longitude 73.46 E, latitude 31.88 N, and altitude 207 m ASL), Punjab, Pakistan (Fig. S1). The climatic zone of the study area is subtropical and semi-arid, with mean annual temperature of 25.3 °C and precipitation of 433.4 mm in the past 6 years. The soil type is clay loam and classifed as Haplic Yermosols (FAO [2014\)](#page-13-13). Before initiating the experiment in 2016, soil samples were taken by using core sampler (5 cm height, 5.05 cm diameter) at a depth of 0–20 cm. Initial soil had 1.37 g kg<sup>-1</sup> SOC, 7.8 pH, 1.32 g cm<sup>-3</sup> bulk density, 0.28 g kg<sup>-1</sup> total nitrogen concentration, 1.35 dSm−1 electrical conductivity, 4.93 mg  $kg^{-1}$  available phosphorus, and 110 mg kg<sup>-1</sup> exchangeable potassium. Rice–wheat is the predominant cropping system in this region.

This site-specifc experiment was based on diferent tillage system that begun in rice season of 2016. The experiment was laid out in randomized compete block design, with three replications. There were 12 plots, and the individual

plot size was  $15 \times 8$  m (120 m<sup>2</sup>). Two tillage and two residue retention methods investigated were CT0 (puddled rice, conventional wheat − residue); CTR (puddled rice, conventional wheat + residue); NT0 (direct rice seeding, zero-tilled wheat − residue); and NTR (direct rice seeding, zero-tilled wheat + residue). Additional details of the experimental treatments are presented in Table [1](#page-2-0).

### **2.2 Soil Sampling and Analysis**

Immediately after harvest of rice and wheat (in November 2020, April 2021, and correspondingly November 2021 and April 2022), samples of soil from 0–15, 15–30, and 30–45 cm depth were taken from fve randomly chosen points. The soil samples were air-dried at room temperature (~20–25°C room temperature) until the moisture from the surface of the clods completely evaporates. The visible parts of crop straw and debris were physically removed. Big clods (greater than 12 mm) were broken by visualizing natural breaks points by putting hand force. Thereafter, samples were sieved by hand using 10-mm sieve.

Sub-samples of 100 g soil were sieved on Motorized Vibratory Sieve Shaker (FRITSCH, Germany) for 3 min to obtain diferent aggregate fractions, i.e., **>** 2 mm (large macro-aggregate), 2–0.25 mm (small macro-aggregate),  $0.25-0.053$  mm (micro-aggregate), and  $< 0.053$  mm (silt–clay particles) using dry sieving technique. A proper sieving duration (3 min) and amplitude (3 mm) were chosen to obtain appropriate aggregate fractions (Sarker et al. [2018b](#page-14-5)). Soil retained on diferent sieves were weighed and then determined associated SOC in these aggregates using  $K_2Cr_2O_7$  oxidation method (Walkley and Black [1934](#page-15-6)). The mean weight diameter (MWD; mm) of the soil aggregates was estimated by using Eq.  $(1)$  $(1)$ :

<span id="page-2-1"></span>MWD = 
$$
\frac{\sum_{i=1}^{n} (Wi \times Xi)}{\sum_{i=1}^{n} Wi}
$$
 (1)

<span id="page-2-0"></span>**Table 1** Details of the experimental treatments under rice–wheat cropping system

	Treatments Tillage and residue management
C <sub>T</sub> O	In CT0, one disc harrowing and one cultivation followed by planking, and then the plots were flooded with irrigation water, and puddling was done with two wet plowing and then planking, followed by manual transplanting of rice seedlings in the soft-mud (puddled soil). After the rice harvesting, wheat was sown on 22.5 cm spaced rows using seed drill after conventional tillage (one disc harrowing, two plowing, and two planking) without residue addition
<b>CTR</b>	The plots under CTR were followed the similar practices as CT0 with 100% residue returning of the preceding crop by using disc harrowing and plow tillage
NT <sub>0</sub>	In NT0, no cultivation was done, direct rice sown (DSR) with multi-crop inclined plate seeding drill (Green Land Engineering Ltd Daska, Pakistan) in 22.5 cm spaced rows without residue addition. After DSR, wheat was sown without residue addition under no-till conditions using happy seeder (Sharif Engineering Ltd Faisalabad, Pakistan) at 22.5 cm spaced rows
<b>NTR</b>	In NTR, all the management practices kept similar to NT0 with 100 % residue retention of the preceding crop. The whole straw was chopped into 5–8-cm-long pieces by tractor driven mechanical chopper

*CT0*/*CTR* indicates puddled rice; conventional wheat without/with residue retention. *NT0*/*NTR* represents direct seeded rice; zero-tilled wheat without/with residue retention

where  $X_i$  indicated the mean aggregate diameter of particular aggregate fraction (mm) and  $W_i$  represented the weight percentage of each aggregate fraction (Pirmoradian et al. [2005](#page-14-10)).

The geometric mean diameter (GMD; mm) was determined according to Eq. ([2\)](#page-3-0):

$$
GMD = \exp\left[\frac{\sum_{i=1}^{n} Wi \times \ln(Xi)}{\sum_{i=1}^{n} Wi}\right]
$$
 (2)

where  $W_i$  represents the weight percentage of the each aggregate fraction and  $X_i$  indicates the mean diameter of the particular aggregate fraction (Meng et al. [2014](#page-14-11)).

### **2.3 Incubation Experiment**

Briefly, 30 g air-dried soil sample of the macro-  $(> 2$  mm) and micro-aggregate  $(< 2$  mm) fraction from 0–15 and 15–30 cm depths was incubated at 70% water holding capacity (WHC) in air-tight mason jars (350 ml) at  $25 \pm 1$  °C over 60 days in the laboratory. All jars were pre-incubated for 7 days at 30°C in the dark and then placed in incubator for 60 days, in triplicate. The WHC was assessed by soaking in wet and dry conditions (Shahbaz et al.  $2017$ ; Kan et al.  $2020a$ ). To trap the released  $CO<sub>2</sub>$ , small beakers (20 ml) containing 10 ml of 1 M of NaOH were replaced at each measurement. The released  $CO<sub>2</sub>$  was trapped in NaOH, which was measured at 3, 5, 7, 15, 30, 45, and 60 days after incubation. Additional carbonates were removed by using  $BaCl<sub>2</sub>$  to convert  $Na<sub>2</sub>CO<sub>3</sub>$  into  $BaCO<sub>3</sub>$ . The remaining NaOH was back-titrated with 0.1 M HCl using phenolphthalein as indicator (Kumar et al. [2018](#page-14-13)). To determine the headspace  $CO<sub>2</sub>$ , blank jars with NaOH solutions (for  $CO<sub>2</sub>$  trap) were also incubated. Cumulative amount of  $CO<sub>2</sub>-C$  evolved during the 60-day incubation period was represented as C mineralization and expressed as mg  $CO<sub>2</sub>$  kg<sup>-1</sup> soil. Moreover, first-order kinetic model was used for the estimation of the decomposition rate of soil C as affected by different tillage systems (Eq.  $(3)$  $(3)$ ):

$$
C_m = Co(1 - e^{-kt})
$$
\n<sup>(3)</sup>

where  $C_m$  indicated the cumulative mineralized  $CO_2$ -C emission (mg  $CO_2$  kg<sup>-1</sup> aggregate) after time t (days); Co indicated the initially mineralizable C; and *k* represents the rate constant.

Carbon mineralizability (labile C proportion) represented as g  $CO_2$ -C g<sup>-1</sup> SOC and calculated according to Eq. ([4\)](#page-3-2) (Das et al. [2019\)](#page-13-14):

$$
C_{\text{mineralizability}} = \frac{Cm}{\text{SOC concentration}} \tag{4}
$$

where  $C_m$  is the total cumulative SOC mineralization (mg  $CO<sub>2</sub> kg<sup>-1</sup> soil/aggregate)$  after 60-day incubation. The SOC

concentration (g  $kg^{-1}$ ) indicates the average SOC contents in aggregate fractions.

<span id="page-3-0"></span>Rice and wheat were manually harvested from  $3 \text{ m}^2$  area from each plot, and the crop biomass was then oven dried at  $60^{\circ}$ C until the constant weight to obtain the adjusted straw yield which was presented after subtracting the moisture content. The total C input considering the above-ground plant parts  $(C_s)$ , below-ground  $(C_r)$ , and rhizodeposition  $(C_{\text{rhizo}})$ were estimated from using empirical method (Zhang et al. [2022\)](#page-15-7). Moreover, the C input estimation by root biomass percentage distribution in the specifc soil layer (0–15, 15–30, and 30–45 cm) under diferent tillage systems was calculated by using the fndings of Alam et al. ([2014](#page-13-15)). The root biomass distribution of rice and wheat crop under diferent tillage systems was estimated (Huang et al. [2011;](#page-13-16) Singh et al. [2014](#page-14-14)). Moreover, the CTR tillage incorporates 10% of their retained residue at 15–30 cm (Mairhofer et al. [2019](#page-14-15)); therefore, 10% C input of residue was included in 15–30 cm soil:

$$
Total C inputs = Cs + Cr + Cthizo
$$
 (5)

The above-ground biomass straw's  $(C_s)$  C inputs were calculated as

$$
C_s = B_{\text{straw}} \times 0.45 \tag{6}
$$

Rice and wheat straw biomass is known as  $B_{\text{straw}}$  (Mg ha<sup>-1</sup>). The crop biomass C input was determined using the above-ground plant parts' 45% C concentration (Johnson et al. [2006\)](#page-14-16).

Below-ground root  $(C_r)$  contribution to the C input was calculated as

<span id="page-3-3"></span>
$$
C_r = B_{straw} \times rs \times 0.45
$$
 (7)

where the root-to-shoot ratio is denoted by rs. The root-toshoot ratios for rice and wheat are approximately 0.13 (Chen et al. [2014](#page-13-17)) and 0.22, respectively (Kong et al. [2005\)](#page-14-17).

The C addition from rhizodeposition  $(C_{\text{rhizo}})$  was estimated using Eq. ([7\)](#page-3-3) as described by Maillard and McConkey ([2018](#page-14-18)):

<span id="page-3-1"></span>
$$
C_{\text{rhizo}} = C_r \times 0.65\tag{8}
$$

#### **2.4 Statistical Analysis**

<span id="page-3-2"></span>Statistical analysis was carried by using the SPSS 20.0 software package (SPSS Inc., Chicago, IL 2004). The signifcant diferences among treatments in soil aggregate size fractions, aggregate associate C, soil structural stability, and cumulative C mineralization were assessed by one-way ANNOVA with the least signifcant diference (LSD) test at  $p < 0.05$ .

## **3 Results**

# **3.1 Soil Aggregate Size Distribution, Structural Stability, and Associated SOC Concentration**

The soil aggregate size distribution varied significantly  $(p <$ 0.05) among diferent tillage systems, and the macro-aggregate fractions were the most abundant in rice and wheat seasons at 0–45 cm soil depths (Fig. [1\)](#page-4-0), following the order  $NTR > CTR > NTO > CTO$ . The higher macro-aggregate fractions were obtained under NTR, which were considerably greater than CTR, NT0, and CT0 in rice season by 7%, 7.9%, and 17.2% at 0–15 cm layer and by 3.5%, 3.8%, and 17.2% at 15–30 cm layer, and by 7.7%, 7.4%, and 16.9% at 30–45 cm layer (Fig. [1](#page-4-0)). A similar trend was observed during the wheat season at 0–45 cm layer (Fig. [2](#page-5-0)).

The increase in macro-aggregate proportion resulted in a signifcant increase in MWD and GMD at 0–45 cm soil depth (Table [2\)](#page-6-0). Diferent tillage systems signifcantly affected the MWD and GMD at  $0-45$  cm layer ( $p < 0.05$ ). Generally, NTR increased the soil structural stability in rice and wheat at all layers (0–45 cm) compared to other treatments. Specifcally, the values of MWD and GMD averagely improved under CTR, NT0, and NTR in rice by 10.7%, 11%, 18.3%, and 41.1%, 42.5%, 72% and wheat by 14.4%, 12.5%, 19.6%, and 45.6%, 39.4%, and 66.6%, respectively, at 0–15 cm layer and 13.3%, 13.3%, 17.8%, and 49%, 48.5%, and 68.5% in rice and 11.9%, 11.4%, 17.7%, and 37%, 36.9%, and 61.1% in wheat at 15–30 cm layer compared with CT0. Similarly, at 30–45 cm layer, the MWD and GMD increased under CTR, NT0, and NTR by 10.5%, 9.6%, 16.8%, and 39.1%, 35.5%, and 64.2% in rice and wheat, compared to CT0 (Table [2](#page-6-0)).

At a depth of 0–30 cm, there were obvious diferences across tillage practices, but after this depth, there were no significant changes in the SOC content for different treatments in the rice and wheat seasons (Table S3). Moreover, the aggregate-associated SOC concentration decreased with increase in soil depth, and the maximum concentration was in 0–15 cm layer (Fig. [3](#page-7-0) and [4\)](#page-8-0). In 0–15 cm layer, the aggregate-associated SOC concentration in rice and wheat seasons varied signifcantly in diferent tillage systems (*p* < 0.05), with the greatest value observed under NTR and the lowest under CT0. Compared with CT0, SOC concentration



<span id="page-4-0"></span>**Fig. 1** Soil aggregate size distribution (%) of diferent aggregate fractions (> 2 mm, 2–0.25 mm, 0.25–0.053, mm and < 0.053 mm) in rice 2020 (**a**, **b**, **c**) and 2021 (**d**, **e**, **f**) from 0–15, 15–30, and 30–45 cm soil depths under diferent tillage systems. CT0/CTR indicates puddled

rice; conventional wheat without/with residue retention. NT0/NTR represents direct seeded rice; zero-tilled wheat without/with residue retention



<span id="page-5-0"></span>**Fig. 2** Soil aggregate size distribution (%) of diferent aggregate fractions (> 2 mm, 2–0.25 mm, 0.25–0.053 mm and < 0.053 mm) in wheat 2020 (**a**, **b**, **c**) and 2021 (**d**, **e**, **f**) from 0–15, 15–30, and 30–45 cm soil depth under diferent tillage systems. CT0/CTR indicates

puddled rice; conventional wheat without/with residue retention. NT0/NTR represents direct seeded rice; zero-tilled wheat without/ with residue retention

in > 2 mm, 2–0.25 mm, 0.25–0.053 mm, and < 0.053 mm increased by 31.1%, 38%, 33.9%, and 34.4% and by 29.1%, 36.2%, 30.6%, and 37.1% under NTR at 0–15 cm layer in rice 2020 and 2021 (Fig. [3a](#page-7-0), d) and by 25.9%, 37.2%, 32.2%, and 37.1 % and by 24.1 %, 34.2 %, 31.8 %, and 36.5 % in wheat 2020 and 2021, respectively (Fig. [4](#page-8-0)a, d). Likewise, a signifcant increase was observed in aggregate-associated SOC in the  $> 2$  mm, 2–0.25 mm, 0.25–0.053 mm, and < 0.053 mm which was 31.7%, 42.2%, 37.3%, 43.3%, and 33%, 36.7%, 34.2%, and 39.4% in rice 2020 and 2021 (Fig. [3b](#page-7-0), e) and by 30.4 %, 40.9 %, 31 %, 37.5 %, and 22.9%, 36.2%, 34.1%, and 37.5% in wheat 2020–2021 at 15–30 cm layer, respectively, under CTR compared with CT0 (Fig. [4b](#page-8-0), e). Different tillage systems did not significantly affect aggregate-associated SOC at 30–45 cm layer, except in rice 2020 (Figs. [3](#page-7-0) and [4](#page-8-0)).

# **3.2 Cumulative SOC Mineralization in Aggregates Under Diferent Tillage System**

During the first 2 weeks of incubation, pronounced  $CO<sub>2</sub>$ emissions were observed that became stable over time (15 to 60 days) (Fig. S2, S3, S4, and S5). Diferent tillage systems

strongly afected the cumulative SOC mineralization in macro- and micro-aggregates over 60 day's incubation (*p* < 0.05). In general,  $CO<sub>2</sub>$ -C emissions were greater at 0–15 cm than that in  $15-30$  cm layer (Fig. [5](#page-9-0) and [6](#page-10-0)). Further, cumulative SOC mineralization rate was higher in macro-aggregates than micro-aggregates. Specifcally, in rice seasons (2020–2021), NTR on average increased 36.2% and 45.5% C mineralization in macro- and micro-aggregates (Fig. [5](#page-9-0)), while 43.2% and 55.4% increase in wheat (2020–2021), respectively, at 0–15 cm layer compared with CT0 (Fig. [6](#page-10-0)). However, at 15–30 cm layer, CTR increased 16.9% and 24.3% SOC mineralization in macro- and micro-aggregates in rice and by 33.7% and 39% in wheat respectively, compared with CT0.

## **3.3 Carbon Mineralization Kinetics**

The first-order kinetic equation/model was used to fit C mineralization data with correlation coefficient  $(R^2 = 0.97 - 0.98)$ (Table [3\)](#page-11-0). The kinetics parameter estimated the C mineralization rate and the constant value for diferent tillage systems at 0–15 and 15–30 cm layer. Overall, higher mineralization rates were noted at 0–15 cm than 15–30 cm layer.

<span id="page-6-0"></span>**Table 2** MWD and GMD from 0 to 45 cm soil profle under diferent tillage system



*CT0*/*CTR* indicates puddled rice; conventional wheat without/with residue retention. *NT0*/*NTR* represents direct seeded rice; zero-tilled wheat without/with residue retention. Lowercase letters shows statistical significant difference ( $p < 0.05$ ) among treatments

Notably, higher C mineralization rate were observed under NTR at 0–15 cm layer, while CTR had higher mineralization rate at 15–30 cm layer in rice and wheat seasons.

0–15 and 15–30 cm depths. Non-signifcant diferences were observed among diferent tillage systems.

## **3.4 Total C Input and C Mineralizability**

Figure S8 and S9 shows that total C input (considering straw, root, and rhizodeposition C) significantly ( $p < 0.05$ ) affected by diferent tillage systems at 0–15, 15–30, and 30–45 cm soil layers in rice and wheat rotation. The treatments under residue return CTR and NTR signifcantly increased the total C input at 0–15 cm soil layer in both rice and wheat seasons, compared to other treatments (Fig. S8a, d and S9a, d). However, CTR signifcantly increased the total C input at 15–30 cm soil layer compared to the other treatments in rice and wheat by followed the trend as  $CTR > CT0 > NTR \geq NTO$ (Fig. S8b, e and S9b, e). Moreover, CTR and CT0 almost equally contributed to total C input at 30–45 cm layer, compared to other treatments (Fig. S8c, f and S9c, f).

Diferent tillage systems did not signifcantly afect the C mineralizability in rice and wheat season at 0–15 and 15–30 cm layer (Table S2). Generally, C mineralizability showed an increasing trend over the period of time. Overall, non-signifcant but higher values of C mineralizability were observed in micro-aggregates than in macro-aggregates at

## **4 Discussion**

Soil aggregation could afect the SOC distribution in the soil profle, which could be driven by diferent tillage and residue inputs (Weidhuner et al. [2021\)](#page-15-8). Results presented herein show that different tillage practices significantly affect the total C input at three soil layers (0–15, 15–30, and 30–45 cm). The residue input under diferent tillage systems can increase the supply of C required for the formation of aggregates (Verhulst et al. [2011\)](#page-15-9). In general, NTR increased the macro-aggregate contents at the surface soil layer (0–15 cm); however, CTR increased at subsurface soil layer (15–30 cm). It is widely reported that conventional plowing (CTR) has a negative impact on the macro-aggregates at upper soil layer (0–15 cm); however, at subsurface soil (below 15 cm soil layer), CTR results in an increased incorporation of fresh organic material and residue due to tine cultivation depth and efficient litter translocation. These processes can induce microbial activity, produce binding agents, and function as a location for the nucleation of macro-aggregates in deeper soil layers (Luo et al. [2010\)](#page-14-19). Moreover, soil aggregation



<span id="page-7-0"></span>**Fig. 3** SOC concentrations (g  $kg^{-1}$ ) in soil aggregates (> 2 mm, 2–0.25 mm, 0.25–0.053 mm, < 0.053 mm) in rice 2020 (**a**, **b**, **c**) and 2021 (**d**, **e**, **f**) from 0 to 45 cm soil profle under diferent tillage systems. CT0/CTR indicates puddled rice; conventional wheat without/

with residue retention. NT0/NTR represents direct seeded rice; zerotilled wheat without/with residue retention. Error bars indicate standard error

was not significantly affected by rice and wheat cropping system. However, the relationship among total C input and soil aggregation was significantly affected by different tillage and residue inputs at diferent soil depths. For example, residue addition in a paddy season decomposed quickly due to higher microbial activity under continuous water application and release several particle binding agents (particulate carbon) that might contribute in soil aggregation process (Wang et al.  $2019$ ). The relationship of total C input with  $> 2$  mm soil aggregation found that C input of NT0, NTR, and CTR increased  $> 2$  mm aggregates at 0–15 cm, while C input of CTR increased  $> 2$  mm at 15–30 cm soil depth. The soil aggregation is mainly driven by particle binding agents and microbial growth such as fungal hyphal network (Hartmann [2022](#page-13-18)). In the same way, NTR might increase fungal hyphal network under residue retention. In contrast, CTR can break hyphal network due to repeated cultivation and thereby decrease aggregation (Roger-Estrade et al. [2010\)](#page-14-20). Similarly, the higher amount of C derived from decomposed residue signifcantly promoted aggregate-associated SOC (Huang et al. [2018\)](#page-13-19). Both wheat and rice crops had similar SOC concentration, i.e., higher SOC in macro-aggregates that decreased with decrease in aggregate class size. This trend may be attributed to the fact that macro-aggregate formation comes from particulate organic matter and other residue derived binding agents (Kravchenko et al. [2015](#page-14-21); Xue et al. [2019\)](#page-15-10). More importantly, this mechanism could be enhanced under wet feld condition coupled with residue addition and minimum tillage (Xue et al. [2019;](#page-15-10) Zhang et al. [2023\)](#page-15-11). Similar results were reported by Kan et al. ([2020a](#page-14-6)) and Wang et al. [\(2019\)](#page-15-5) for wheat and rice feld of north China plains and southern China, respectively. Some studies have emphasized that long-term conventional tillage practices destroy soil structure mechanically as well as fungal hyphae structure, resulting in lower proportion of macro-aggregates in the feld. In addition, results presented herein show that soil stability indicators such as MWD and GMD had higher values under residue return and conservation tillage treatments than those under conventional tillage. Similarly, conservation tillage practices are known to improve soil structure and stability not only in the surface layer, but also in sub-soil, probably due to greater microbial abundance and earthworm



<span id="page-8-0"></span>**Fig. 4** SOC concentrations (g kg<sup>-1</sup>) in soil aggregates (> 2 mm, 2–0.25 mm, 0.25–0.053 mm, < 0.053 mm) in wheat 2020 (**a**, **b**, **c**) and 2021 (**d**, **e**, **f**) from 0 to 45 cm soil profle under diferent tillage systems. CT0/CTR indicates puddled rice; conventional wheat with-

out/with residue retention. NT0/NTR represents direct seeded rice; zero-tilled wheat without/with residue retention. Error bars indicate standard error

biomass, which greatly contribute to stabilize soil structure (Table [4\)](#page-12-0) (Bhattacharyya et al. [2021\)](#page-13-20).

Soil C mineralization was higher in the aggregates from upper soil layer that decreased with increase in soil depth in both crops. In terms of tillage comparisons, NTR and CTR had higher C mineralization at 0–15 cm soil depth as compared to NT0 and CT0. These results depicted that conservation and conventional tillage systems without residue addition had lower SOC contents that might reduce C mineralization. In contrast, however, NTR and CTR had higher C input in the form of residue, resulting in higher SOC storage and C mineralization (Kan et al. [2020a](#page-14-6); He et al. [2023](#page-13-21)). In fact, C input and loss are a major problem in most regions of the world. Such C sequestration and stabilization could be the main strategy to mitigate climate change (Lal [2004](#page-14-0)). The cumulative C mineralization was higher in  $> 2$  mm aggregates, suggesting that higher macro-aggregates had relatively higher SOC concentration as compared to microaggregates (Kan et al. [2020a\)](#page-14-6). Specifcally, the rice season sampled aggregates had relatively higher C mineralization as compared to those obtained during the wheat season in both years. The plausible explanation of this phenomena is that rice crop has continuous or frequent water supply that could decompose crop residue and provide instant material to microbes for processing/oxidation (Qi et al. [2021](#page-14-22)). Several studies have elaborated that higher concentration of SOC along with organic acids could enhance microbial activities and C oxidation (Zhang et al. [2021\)](#page-15-12). Soil structure is also a major indicator infuencing C mineralization, and soil structure is afected by agronomic and tillage management practices (Raiesi and Kabiri [2017;](#page-14-23) Guo et al. [2019\)](#page-13-22). In general, macro-aggregates had higher C mineralization at 0–15 cm soil depth as compared to 15–30 cm soil depth. This trend shows that macro-aggregates at upper soil depth had higher proportion of C input in the form of crop residue because surface residue application is efective in upper soil layer (Zhang et al. [2022](#page-15-7)). Another plausible explanation of this result is that the higher proportion of macro-aggregates was



<span id="page-9-0"></span>**Fig. 5** Cumulative C mineralization rate (mg  $CO_2$  kg<sup>-1</sup> soil) in soil aggregates  $> 2$  mm (**a**, **c**) (macro-aggregates) and  $< 2$  mm (**b**, **d**) (micro-aggregates) under diferent tillage practices at 0–15 and 15–30 cm soil profle in rice seasons 2020–2021. CT0/CTR indicates puddled rice; conventional wheat without/with residue retention. NT0/

NTR shows direct seeded rice; zero-tilled wheat without/with residue retention. Diferent lowercase letters indicate statistical signifcant difference  $(p < 0.05)$  among treatments. Error bars indicate standard error. NS, non-signifcant

present at upper soil layer that decreased with increase in soil depth. Therefore, higher proportion of macro-aggregates contains higher amount of carbon as compared to lower proportion of macro-aggregates (Andruschkewitsch et al. [2014](#page-13-3)). In addition, residue-added tillage treatments had relatively higher cumulative C mineralization rate as compared to residue removal tillage treatments; this might be due to residue addition and tillage treatment diferences (Kan et al. [2020c](#page-14-24); Virk et al. [2021\)](#page-15-13). Several studies have reported that plow tillage and no tillage may have higher cumulative C mineralization or oxidation process in the feld conditions right after tillage implementation, because continuous application of tillage practices disrupt soil aggregation, exposing physically protected SOC and make it available to microbial oxidation (Kan et al. [2020b](#page-14-25); Liu et al. [2022](#page-14-26)). Despite a higher C mineralization in residue-added tillage treatments, residue addition and conservation agricultural practices can decrease C mineralization potential when comparing with conventional tillage practices (Datta et al. [2019](#page-13-23); Kan et al. [2020a\)](#page-14-6). Moreover, conservation tillage practices mostly favor soil structural stability and aggregation, increasing SOC sequestration and stability as compared to conventional tillage practices (He et al. [2023\)](#page-13-21). Kan et al. [\(2020a](#page-14-6)) and Qi et al. [\(2021](#page-14-22)) also reported that SOC mineralizability (mg of oxidize  $CO<sub>2</sub>$  per g of SOC) is another important indicator to evaluate the SOC stabilization in bulk soil or aggregates,



<span id="page-10-0"></span>**Fig. 6** Cumulative C mineralization (mg  $CO<sub>2</sub>$  kg<sup>-1</sup> soil) in soil aggregates  $> 2$  mm (**a**, **c**) (macro-aggregates) and  $< 2$  mm (**b**, **d**) (microaggregates) under diferent tillage practices at 0–15 and 15–30 cm soil profle in wheat 2020–2021. CT0/CTR indicates puddled rice;

conventional wheat without/with residue retention. NT0/NTR shows direct seeded rice; zero-tilled wheat without/with residue retention. Lowercase letters shows statistical significant difference ( $p < 0.05$ ) among treatments. Error bars indicate standard error

which have been lower in conservation agricultural practices (Duan et al. [2022\)](#page-13-24).

Globally, conservation tillage has been covered 122–215 M ha of the land area, due to its ability to store C (Lal [2004](#page-14-0); Prestele et al. [2018\)](#page-14-27). Conservation tillage significantly influences the aggregate size distribution and aggregate-associated SOC concentration, compared to conventional tillage. However, it is not well-known how SOC sequestered in various aggregates (Zhao et al. [2015\)](#page-15-4). Our results signify that no tillage with residue retention increased the SOC accumulation while decreased the mineralization in macro-aggregates. Briefy, this study demonstrated the importance of macroaggregates encapsulating SOC, thus improving soil quality under NTR. Nevertheless, the mineralization of SOC under feld conditions cannot be evaluated; therefore, laboratory incubation is necessary to estimate the mineralization rate in diferent aggregates. In comparison to our results, others studies also reported higher macro-aggregate fraction and aggregate-associated C under conservation tillage compared to conventional tillage in the North China Plain (Zhang et al. [2017](#page-15-14); Gao et al. [2019](#page-13-25)). Furthermore, the macro-aggregates and associated C were increased under conservation tillage in many parts of the world, e.g., Spain (Hontoria et al. [2016](#page-13-26)), France (Bottinelli et al. [2017\)](#page-13-7), and India (Somasundaram et al. [2017](#page-15-1), [2018](#page-14-3)). On contrary, conservation tillage did not enhance macro-aggregates and associated C stock compared to conventional tillage in Western Australia (Sarker et al. [2018a\)](#page-14-4). Thus, it is suggested that conservation tillage would

	Treatment	$0-15$ cm			15-30 cm		
Season		Mineralization constant $(K)$	C mineralization rate (mg $CO_2-C$ kg <sup>-1</sup> day <sup>-1</sup> )	$R^{\overline{2^*}}$	Mineralization constant $(K)$	C mineralization rate (mg $CO_2-C$ kg <sup>-1</sup> day <sup>-1</sup> )	$R^{\overline{2^*}}$
<b>Rice 2020</b>	C <sub>T</sub> O	0.0168	$11.49 \pm 0.39$	0.978	0.0167	$10.96 \pm 0.38$	0.977
$> 2$ mm	<b>CTR</b>	0.0163	$16.40 \pm 0.60$	0.976	0.0166	$13.50 \pm 0.47$	0.977
	${\rm N} {\rm T} 0$	0.0163	$13.48\pm0.47$	0.977	0.0166	$12.05\pm0.42$	0.977
	<b>NTR</b>	0.0161	$18.99 \pm 0.72$	0.975	0.0167	$12.97 \pm 0.46$	0.977
<b>Rice 2020</b>							
$<2~\rm{mm}$	CT0	0.0171	$10.43 \pm 0.35$	0.978	0.0167	$9.19 \pm 0.32$	0.977
	<b>CTR</b>	0.0170	$15.62 \pm 0.58$	0.974	0.0166	$12.60 \pm 0.44$	0.977
	NT0	0.0169	$13.06 \pm 0.47$	0.976	0.0167	$10.88 \pm 0.38$	0.977
	<b>NTR</b>	0.0164	$17.88 \pm 0.67$	0.975	0.0166	$11.74 \pm 0.41$	0.977
<b>Rice 2021</b>							
$> 2$ mm	CT0	0.0164	$15.68 \pm 0.57$	0.98	0.0164	$11.30 \pm 0.39$	0.977
	<b>CTR</b>	0.0162	$18.12 \pm 0.67$	0.975	0.0165	$14.10 \pm 0.50$	0.977
	NT <sub>0</sub>	0.0165	$16.98 \pm 0.63$	0.975	0.0167	$12.55 \pm 0.44$	0.977
	<b>NTR</b>	0.0159	$20.47 \pm 0.79$	0.974	0.0168	$13.35 \pm 0.47$	0.977
<b>Rice 2021</b>							
$< 2$ mm	CT0	0.0167	$13.86 \pm 0.52$	0.975	0.0168	$10.25 \pm 0.36$	0.977
	<b>CTR</b>	0.0164	$17.45 \pm 0.66$	0.974	0.0166	$13.48\pm0.48$	0.976
	NT <sub>0</sub>	0.0167	$15.59 \pm 0.59$	0.974	0.0166	$11.30\pm0.40$	0.977
	$\ensuremath{\text{NTR}}$	0.0158	$19.70 \pm 0.76$	0.974	0.0166	$12.22 \pm 0.43$	0.977
Wheat 2020							
$> 2$ mm	CT0	0.0169	$13.02 \pm 0.43$	0.978	0.0165	$10.77 \pm 0.35$	0.98
	<b>CTR</b>	0.0166	$18.52\pm0.68$	0.975	0.0164	$18.43\pm0.71$	0.974
	NT <sub>0</sub>	0.0167	$15.87\pm0.56$	0.977	0.0167	$13.71 \pm 0.48$	0.977
	<b>NTR</b>	0.0164	$20.49 \pm 0.77$	0.975	0.0166	$16.25 \pm 0.60$	0.975
Wheat 2020							
$< 2$ mm	CT <sub>0</sub>	0.0161	$11.16 \pm 0.36$	0.979	0.0166	$9.78 \pm 0.32$	0.98
	<b>CTR</b>	0.0165	$16.85 \pm 0.62$	0.975	0.0166	$17.83 \pm 0.69$	0.974
	$\rm{NT}0$	0.0167	$14.13 \pm 0.50$	0.977	0.0167	$12.83 \pm 0.45$	0.977
	<b>NTR</b>	0.0163	$19.31 \pm 0.73$	0.975	0.0167	$15.48\pm0.57$	0.975
Wheat 2021							
$> 2$ mm	CT <sub>0</sub>	0.0165	$12.98\pm0.42$	0.979	0.0167	$10.95 \pm 0.36$	0.979
	<b>CTR</b>	0.0165	$18.96 \pm 0.69$	0.976	0.1637	$18.79 \pm 0.71$	0.975
	${\rm N} {\rm T} 0$	0.0167	$16.49\pm0.58$	0.977	0.0167	$14.07\pm0.49$	0.977
	$\ensuremath{\text{NTR}}\xspace$	0.0169	$23.59 \pm 0.97$	0.971	0.0166	$16.47 \pm 0.61$	0.975
Wheat 2021							
$< 2$ mm	$\ensuremath{\mathbf{CT0}}$	0.0171	$10.96\pm0.36$	0.979	0.0168	$9.99 \pm 0.32$	0.979
	<b>CTR</b>	0.0162	$16.99\pm0.61$	0.976	0.0164	$18.12 \pm 0.69$	0.974
	NT <sub>0</sub>	0.0167	$14.44\pm0.51$	0.977	0.0166	$12.96 \pm 0.45$	0.977
	$\ensuremath{\text{NTR}}$	0.0163	$20.47 \pm 0.78$	0.974	0.0165	$15.60 \pm 0.57$	0.976

<span id="page-11-0"></span>**Table 3** Parameters of first-order exponential equations describing the C mineralization rate (mg CO<sub>2</sub>–C kg<sup>-1</sup> day<sup>-1</sup>) in macro- (> 2 mm) and micro-aggregates (< 2 mm) from 0 to 30 cm soil profle under diferent tillage system

*CT0*/*CTR* shows puddled rice; conventional wheat without/with residue returning. *NT0*/*NTR* shows direct seeded rice; zero-tilled wheat without/ with residue returning

be a viable option to enhance macro-aggregation and associated C stock in most regions of the world.

reveal the mechanism of aggregate formation and C sequestration under diferent tillage systems. Prior studies also showed that C mineralization (from laboratory incubation) and C loss (from feld based experiment) were correlated

A feld experiment and laboratory-based incubation in various aggregates by using dry sieving were combined to

<span id="page-12-0"></span>



*CT0*/*CTR* shows puddled rice; conventional wheat without/with residue returning. *NT0*/*NTR* shows direct seeded rice; zero-tilled wheat without/ with residue returning. \* indicates  $p \le 0.05$ 

(Sierra and Desfontaines [2018\)](#page-14-28). However, the environmental variables (moisture and temperature) can considerably infuence SOC accumulation and mineralization in aggregates (Qi et al. [2019\)](#page-14-29). Moreover, this study exhibits certain drawbacks regarding dry sieving of soil. Unlike wet sieving, clods could not dispersed naturally during dry sieving, and we used external hand force to break the big clods by visualizing their natural breaks points. In this study, the laboratory incubation of SOC mineralization was performed under a constant moisture and temperature. Future studies are needed to examine how laboratory incubation of SOC mineralization responds to environmental elements (e.g., varying moisture and temperature conditions) under diferent tillage practices.

# **5 Conclusions**

The study proved the hypothesis that the retention of crop residues under no tillage (NTR) increases the macroaggregate fraction (> 2 mm) and soil organic carbon (SOC) content in macro-aggregates in a rice–wheat double cropping system. In specifc, higher proportion of macroaggregate fraction was obtained under NTR, which were considerably greater than CTR (conventional tillage with residue return), NT0 (no tillage without residues), and CT0 (conventional tillage without residues) in rice and wheat season, respectively by 7%, 7.9%, and 17.2% and by 5.8%, 6.9%, and 12.1% at 0–15 cm soil layer. Subsequently, higher aggregate-associated SOC was accumulated at 0–15 cm soil layer in macro-aggregates under NTR, compared to other treatments, which considerably

improved the soil structural stability. The relationship of total carbon input with soil aggregation (mainly  $> 2$  mm) followed the pattern of  $NT0 > NTR > CTR > CTO$  at 15–30 cm soil depth. The macro-aggregates under NTR resulted in the highest cumulative carbon mineralization rate at 0–15 cm; however, CTR enhanced the cumulative carbon mineralization at 15–30 cm depth. Overall, it is concluded that NTR is an efective management practice to improve the macro-aggregation, soil structural stability, and SOC contents in a rice–wheat double cropping system. However, further research about microbial contribution in soil aggregation and SOC mineralization should be explored in conservation agricultural practices.

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**Data availability** The data that support the fndings of this study are available on reasonable request.

## **Declarations**

**Competing Interests** The authors declare no competing interests.

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