

# Impact of Conservation Agriculture and Cropping System On Soil Organic Carbon and Its Fractions in Alluvial Soils of Eastern Gangetic Plains

#### Rakesh S

Department of Soil Science and Agricultural Chemistry

#### Abhas K Sinha

Department of Soil Science and Agricultural Chemistry

#### Mahesh Kumar Gathala ( m.gathala@cgiar.org)

International Maize and Wheat Improvement Center Bangladesh https://orcid.org/0000-0001-8282-2953

#### Neal W Menzies,

Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya

#### Sudarshan Dutta

African Plant Nutrition Institute (APNI)

#### Ram C Dalal

The University of Queensland

### Prabir Mukhopadhyay

Department of Soil Science and Agricultural Chemistry

#### Apurba K Chowdhury

Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya

### Prateek M Bhattacharya

Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya

#### Parthendu Poddar

Department of Agronomy, Uttar Banga Krishi Viswavidyalaya

#### Research Article

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### **Abstract**

# **Purpose**

A conservation agriculture-based sustainable intensification (CASI) practices have been proposed as a potential alternative management strategy for achieving the food, water and energy security while sustaining the soil health and climate resilience. In this study, we evaluate the performance of CASI technologies under two cropping systems on carbon (C) dynamics in the soils of recent and old alluvial nature of West Bengal in Eastern Alluvial Ganga Plains.

### **Methods**

The on-farm field study was undertaken for four years during 2014-15 to 2018-19 with an objective of long-term setup at Coochbehar and Malda districts, West Bengal (subtropical eastern India). The two cropping systems (rice-wheat, RW and rice-maize, RM) and two tillage options (zero tillage, ZT and conventional tillage, CT) were evaluated on multi-location at farmers' field to see the impact on soil total organic carbon (TOC) and its fractions, stratification, and stocks.

### **Results**

About 20% higher TOC concentration was observed in the old alluvial soils (Inceptisols of Malda district) as compared to recent alluvial soils (Entisols of Coochbehar district). TOC and its fractions significantly (p<0.05) improved under RM cropping system than that under RW. The ZT system enhanced the TOC and its fractions by 16.8 and 9.8 % over CT at 0–5 and 5–10 cm respectively. All the C fractions showed strong positive correlation (r= >0.85; p < 0.01) with TOC except POC.

# **Conclusions**

Our research indicated that ZT system increased the C turnover rate in both soil types which was found more prominent in RM system.

# **Highlights**

- Conservation agriculture improves soil organic carbon (C)concentration mainly in the topmost soil depth.
- · Addition of residue biomass enhances the soil organic C status as well as its fractions.
- Zero-tillage (ZT) under rice-maize (RM) system increases the soil sequestration of C due to higher biomass addition as compared to rice-wheat (RW).
- Stabilization of C and its fractions reflected more under heavier soils.

Soil organic C fractions are more stratified in clayey soils than sandy soils.

### Introduction

The growing concern of global warming and climate change impacts on the community have spurred interest in enhancing the sequestration of atmospheric carbon dioxide (CO<sub>2</sub>) in terrestrial ecosystems (Dolman et al., 2004; Lal, 2015; Sarkar et al., 2020). According to the Intergovernmental Panel on Climate Change (IPCC), about 22% of global anthropogenic greenhouse gas (GHG) emissions are contributed by agriculture, forestry, and/or other land uses (IPCC, 2019). Cultivation of arable lands leads to the substantial loss of soil organic matter (SOM) and increase emissions of CO2 from soil to the atmosphere, thereby increasing the CO<sub>2</sub> concentration in the atmosphere (Ladha et al., 2015). The SOM is made up of dead plant residues, particulate organic C (POC), humus C, and recalcitrant C. It plays a major role in maintaining the fertility, productivity, and overall quality of soil (Larson and Pierce, 1994; Kang et al., 2005), besides having other important environmental functions (Fageria, 2012). The relative proportion of these fractions reflects in the soil ecosystems, including agricultural and non-agricultural soils, which can directly impact the microbial activity and C dynamics in soil. The labile C fractions in soil are the important component that determines the soil quality Although these fractions constitute a relatively smaller fraction of TOC and have a very short turnover times in soil which are highly sensitive to land management changes (Weil and Magdoff, 2004; Duval et al., 2018). The composition of these C fractions varies depending on the stage of decomposition, but they have critical role in soil functioning and health (Belay-Tedla et al., 2009).

Improved land management practices should not only increase TOC stock, but ideally would optimize the proportion of C in these various TOC fractions. Any system that produces rich source of organic material, will have greater amounts of residue SOC. Thus, the study of TOC has increasingly focussed on identifying fractions of TOC that are related to how labile the C is. Fractions such as hot-water soluble C (HWEC), POC, and mineral associated organic C (MAOC) are used because they are indicative of residence or turnover times (Rakesh et al., 2020). These parameters also have been used as indicators for soil quality (Cambardella and Elliott 1992; Blair et al., 1995; Bolinder et al., 1999; Duval et al., 2018). Distribution of TOC-fractions and their stocks, in the soil profile, or C stratification, helps in identifying the variations in the quality of SOM of topsoil (Álvarez et al., 2011; Zhao et al., 2015).

Conservation agriculture-based sustainable intensification (CASI) management practices involving minimum soil disturbance, efficient crop rotations, and increased crop residue retention provides a means of increasing TOC (Johansen et al., 2012; Sharma et al., 2019). Tillage and residue management may influence C sequestration, microbial activity, and also play an important role in affecting the soil physicochemical and biological properties (Jat et al., 2019; Choudhary et al., 2018). Crop residues have numerous beneficial effects as these are not only a source of organic C and nutrients (Yadvinder et al., 2009) but also form a mulch that conserves the soil moisture (Aulakh et al., 2012; Gathala at al., 2017; Sarkar et al., 2020). Moreover, it is estimated that the application of best management practices in

agriculture has the potential of offsetting GHG emissions in the range of 1.1-4.3 Gt  $CO_2$ -e yr<sup>-1</sup> (UNEP, 2013). Thus, CA practices have the potential to reduce the 10 to 60 percent  $CO_2$ -e emissions over energy intensive conventional systems, depending on the layering of CA practices implemented (Ladha et al., 2015; Gathala et al., 2020; Jat et al., 2020). Addition of organic materials to agricultural soil is important for replenishing the annual C losses and for improving both the biological and chemical properties of the soils (Goyal et al., 1999; Choudhary et al., 2018). Zero tillage (ZT) for crop production has been identified as an important practice to increase soil aggregation and C sequestration (Six et al., 1998; Wright and Hons, 2005; Gathala et al., 2011; Jat et al., 2019) as compared with conventional tillage (CT) by reducing aggregate disruption and the contact between soil microorganisms and organic matter, as well as increasing fungal growth and hyphae that contribute to the formation of large aggregates (Beareet al., 1994; Choudhary et al., 2018).

An understanding of the dynamics of TOC as affected by farming practices is imperative for maintaining soil productivity and mitigating global warming. Adoption of CA among the farmers of alluvial soils of subtropical eastern India, has resulted in increased smallholder profitability, reduced environmental externalities, and improved soil health under various rice-based cropping systems such as rice—wheat (RW), rice—maize (RM), and rice-lentil systems, and their intensification (Islam et al.,2019; Sinha et al., 2019; Gathala et al., 2020). The present study was undertaken to assess the effect of different tillage and crop residue management practices on soil C fractions. The investigation was conducted in selected farmers' fields of an on-going ACIAR-SRFSI research project which was initiated in 2013 to demonstrate the benefits of CA over the conventional system.

We hypothesized that changes in tillage and crop establishment techniques, along with crop residue retention and management practices under different cropping systems, may have a differential impact on the accumulation, and distribution of TOC and different TOC fractions in soil at different depths. Furthermore, that soils (old alluvial soils i.e., Inceptisols of Malda and recent alluvial soils i.e., Entisols of Coochbehar district) with different physicochemical characteristics may not have uniform response to tillage, crop residue retention, and cropping systems. The overall objective of the present investigation was to assess the impact of tillage (ZT and CT) in terms of C distribution in the soil of RW and RM cropping systems. The specific objectives were: (i) to assess the response of different tillage practices and cropping systems on TOC and its fractions after four years (eight seasons) of cultivation, (ii) to explore the stratification of TOC and its fractions at different soil depths, and (iii) to determine the relationships between TOC and its fractions with soil properties in two different agro-ecological regions.

### **Materials And Methods**

# **Description of field sites**

The study was conducted in selected farmer's fields spread across the two districts, Coochbehar (26.3452° N, 89.4482° E) and Malda (25.0108° N, 88.1411° E) of the northern alluvial plains in West Bengal, sub-tropical eastern India. These districts present different soil and edapho climatic conditions.

Field experiments were initiated in 2014-15 with RW and RM cropping systems, for 4 years until 2018-19, altogether 3 rice and 4 wheat or 4 maize crops were grown. These cropping systems were selected on the basis of the existing cropped area, as well as the potential of these systems for improvement of farming livelihoods (Dutta et al., 2020; Mitra et al., 2019; Gathala et al., 2020). This study formed part of an ongoing larger research project entitled 'Sustainable and Resilient Farming System Intensification (SRFSI)' being maintained by the Uttar Banga Krishi Viswavidyalaya (UBKV) in collaboration with the Australian Centre for International Agricultural Research (ACIAR), and the International Maize and Wheat Improvement Centre (CIMMYT) since 2013. The distance between the two field sites / districts was approximately, 400 km. The field sites selected for this study were historically used for growing rice in rotation with other dry-season crops using intensive tillage practices.

The study area has an overall warm humid subtropical climate with unimodal monsoonal rainfall, moderate to hot summer and cold winter, although wide variations existed between the Coochbehar and Malda districts with respect to annual precipitation and air temperature. The Coochbehar site receives a mean (30-year average) annual rainfall of 2357 mm and maximum temperature of 28.2°C and minimum of 20.0°C, while the Malda site has a mean annual rainfall of 1358 mm and maximum temperature of 30.6°C and minimum of 20.2°C.

The experimental trials were conducted as on-farm participatory trials (backed by researchers and managed by farmers; Islam et al., 2019). A total of seven field experimental sites were identified, three (3) in Coochbehar and four (4) in Malda. At each of these field experimental sites collaborating farmers were identified, and a factorial experiment of two cropping systems (RW and RM) and two tillage practices (ZT and CT) with three replications was established to study the effect of CASI practice on C dynamics. Due to the small land area available to each individual farmer, each trial was distributed across six farmer's fields, with each farmer implementing the two tillage practices (ZT and CT) on one cropping system (either RW or RM). Thus the full experiment was a 2x2 factorial conducted at seven sites and replicated three times. The soils at the Coochbehar sites are on recently deposited alluvium Entisol, acidic in reaction, with a light sandy texture (Sarkar et al., 2017). In contrast, the soils of Malda sites are on old alluvial material Inceptisol, neutral to alkaline in reaction, and silty loam to clay loam in texture. The pH (1:2.5 H<sub>2</sub>O), TOC, total nitrogen(N), soil texture, and bulk density of the experimental soils are given in Table 1. The detailed profile description of the studied sites of Malda and Coochbehar along with their taxonomic characteristics (based on NBSS & LUP soil classification, 2001) have been presented in supplementary table S1.

Table 1 Soil pH, total organic C, total N, texture and bulk density (0-20 cm) of the experimental sites

District	Field site (FS)	рН	Total organic carbon	Total- N				Bulk density
			(g kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	Sand (%)	Silt (%)	Clay (%)	(Mg m <sup>-3</sup> )
Malda	FS1	8.47	8.83	1.32	18	66	16	1.37
	FS2	6.82	7.75	1.26	35	46	19	1.31
	FS3	7.44	14.98	1.33	42	43	15	1.33
	FS4	6.80	12.13	1.55	8	64	28	1.45
	Mean	7.02	11.62	1.38	28	51	20	1.36
Coochbehar	FS5	6.42	9.88	1.01	29	58	13	1.05
	FS6	5.91	8.85	0.93	28	62	10	1.11
	FS7	5.56	10.58	0.74	28	60	12	1.17
	Mean	5.96	9.77	0.89	28	60	12	1.11

# Crop and management practices

Wheat and maize crops were sown as early as possible after the harvest of the rice crops to capture the residual soil moisture. The sowing dates varied across the farmer fields, the field sites (FS) and the districts, from the first week of November to the last week of December, depending on the time of harvest of the rice crop. The detailed management practices are presented in Islam et al. (2019). An individual cropping system (RW and RM), consisted of two tillage treatments (CT and ZT) was established at all 42 (each 21 for RM and RW) farmers' fields in the seven selected field sites (FS) of Malda and Coochbehar; these trails were maintained for the term of the experiment and the area under each treatment plot was 666 m<sup>2</sup> (0.07ha). The tillage and cropping systems used for CT were: Puddled transplanted rice (PTR) – CT maize or wheat; and ZT: Unpuddled transplanted rice (UPTR) - ZT maize or wheat. In CT-PTR, two to three dry tillage operations were followed by cross operation of wet-tillage before rice transplanting; for CT winter dry season crops (wheat and maize), fields were prepared with two-three tillage operations followed by laddering. Seedlings of rice were transplanted at 22 cm row spacing in the ZT using a mechanical transplanter, and planted randomly by hand in the CT resulting in 28-30 hill/m<sup>2</sup>. Wheat was sown at 20 cm row spacing in the ZT with continuous seeding (180-200 plant/m<sup>2</sup>) and broadcasted in the CT. Maize was planted at 60 × 20 cm (row × plant) in both the ZT and CT resulting in 75000-80000 plants/ha. Crops were fertilized at rates (kg ha<sup>-1</sup>) recommended for the area; rice 80-90 N, 15-20 P, 40-70 K; wheat 125-145 N, 20-25 P, 40-60 K; and maize 155-180 N, 20-25 P, 60-75 K. Detailed agronomic practice for each treatment are available in Islam et al. (2019).

# Collection of soil samples

Before the start of the experiment, soil samples (0–20 cm) were collected from each experimental site for the determination of the initial physicochemical properties of the soils. The samples were collected from 5 to 6 random spots in an individual farmer's field using an auger of 5 cm diameter and mixed thoroughly to form a composite sample; three composite samples from each field site were collected. The soil samples were air-dried and ground to pass through 2 mm sieve (for analysis of soil properties) and a subsample was further ground to pass through 0.5 mm sieve (for determination of soil organic C). Processed soil samples were stored in sealed polythene containers till analyses were completed.

After the final harvest of wheat (April, 2018-19) and maize (May, 2018-19) crops at the end of 4 years of the experiment, soil samples were collected at 0-5, 5-10 and 10-20 cm depths from each plot in each field, with 5-6 random spots, mixed to one composite sample for each soil depth. Air dried composite samples were ground to pass through 2mm sieve for general analysis and a portion ground to pass through 0.5 mm sieve for SOC and its fractions and kept in sealed polythene containers until analyses were completed.

# Soil analysis

# Soil properties

The pH of soil suspension in a soil: water ratio of 1:2.5 was determined with a pH meter, as described by Jackson (1967). Bulk density (BD) of soil samples was estimated using a core sampler of dimensions 5 × 5 cm (height × diameter) following the method of Cresswell and Hamilton (2002). The proportion of sand, silt, and clay in soil samples was determined by the Bouyoucos hydrometer method (Bouyoucos, 1962). The texture of the soils was ascertained from the particle-size distribution of sand, silt, and clay using soil texture triangle.

# **Carbon fractions**

### Total organic C (TOC)

A modified Walkley and Black method (Baker, 1976) was followed for the analysis of TOC in soil determined by colorimetric method using sucrose as a standard. Briefly, one gram of soil sample was digested in the presence of 20 ml of  $5\% \text{ K}_2\text{Cr}_2\text{O}_7$  and 10 mL of concentrated  $\text{H}_2\text{SO}_4$ . After cooling for 30 minutes, 50 mL of  $0.4\% \text{ BaCl}_2$  was added and allowed to stand overnight. The intensity of the yellow/orange colour was read at 600 nm wavelength using a UV-visible spectrophotometer.

### Hot water extractable C (HWEC)

HWEC was determined by hot water extraction method (Ghani et al., 2003). The air-dried soil sample of 3 g was weighed into 50 mL centrifuge tube, 30 mL of de-ionized water was added, and the suspension was shaken for 30 minutes at 30 rpm and at room temperature. Then, it was centrifuged for 20 minutes

at 3000 rpm, thereafter the supernatant was discarded to remove the cold-water soluble C. A further 30 mL of de-ionized water was added to the same residue and placed on a hot water bath at 80°C for 16 hours. After cooling down, the tubes were then shaken and centrifuged at 3000 rpm for 20 minutes. The supernatant was filtered through cellulose nitrate membrane (0.45  $\mu$ m). The C concentration in the extract was determined by Nelson and Sommers (1982) method. A 4 mL of sample was oxidised with 1 mL of 0.066 M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> at 150°C for 30 minutes. Samples after cooling, were titrated against 0.033 M ferrous ammonium sulphate with 2–3 drops of o-phenanthrolene indicator until the colour turned from greenish violet to brick red.

#### Particulate Organic C (POC)

For the POC fraction (Cambardella and Elliott. 1992), 25 g of air-dried soil was dispersed in 100 mL of 0.5% sodium hexa-metaphosphate in reciprocating shaker for 16 hours. Suspension was then passed through 0.53 mm sieve followed by washing with de-ionised water to collect the >0.53 mm, the POM remained on the sieve. The POM was then dried and powdered. The C concentration of the POM was determined by following the modified Walkley and Black method (Baker, 1976).

#### Mineral associated Organic C (MOAC)

MOAC (<0.53 mm) was calculated by subtracting POC from TOC.

MAOC = TOC - POC

# Stratification ratio (SR)

The stratification ratio of a soil property is defined as the ratio of its value at the soil surface to that at a lower depth (Franzluebbers, 2002). This ratio for a C fraction for 0-10 cm depth was calculated by dividing its value at 0-5 cm to that of its 5-10 cm depth. Similarly, for 0-20 cm depth, the value of 0-5 cm depth was divided by its C concentration at 10-20 cm soil depth.

# Soil Organic C Stock

The C stock in soil was calculated considering soil depth (m), bulk density (BD, Mg m<sup>-3</sup>) and concentration (%) of TOC fraction using the following equation. There was no gravel in the soil samples at any of the sites.

TOC stock (Mg  $ha^{-1}$ ) =  $10^4 ha^{-1} \times BD$  (Mg  $m^{-3}$ ) × soil depth (m) × TOC fraction (%) x  $10^{-2}$ 

# Data analysis

Prior to performing statistical analysis, the normality assumption of analysis of variance (ANOVA) was tested using Shapiro-Wilk test (1965) using JMP statistical software (V9 software, Buckinghamshire, UK). Since the normality assumption of ANOVA was met, the data were not transformed. The data were analysed using proc GLM (general linear model) in SAS. We considered district (D), cropping system (CS), tillage treatments (T) and their interactions (CS x T; D x CS; D x T; D x CS x T) as fixed effects and farmer

(replication) as a random effect in the fit-ANOVA model. The three-way interaction (D x CS x T) were not significant for any of the parameters at any depths except for TOC, MAOC, BD and TOC stock observed at 10-20 cm soil depth. The treatment means for all parameters were compared using Tukey's honest significant difference (HSD) test.

As the soil depth interval is a non-randomized factor, a mixed procedure with repeated measures was used for each experiment and analysed separately for each site. A correlation test was performed to determine correlations among soil organic C fractions with key important soil attributes at 0-5, 5-10, and 10-20 cm depths at the Malda and Coochbehar sites.

### Results

Effect of cropping system and tillage on the concentrations of total organic C(TOC), hot water extractable C (HWEC), particulate organic C (POC) and mineral associated organic C (MAOC)

TOC concentration varied widely between experimental sites and districts (Table 2).

Table 2
Effect of environment (District), cropping systems and tillage on total organic C (TOC) and hot water extractable C (HWEC) concentrations at different soil depths.

	TOC concentration (g kg <sup>-1</sup> )			HWEC conc	HWEC concentration (mg kg <sup>-1</sup> )				
	0-5 cm	5-10 cm	10-20 cm	0-5 cm	5-10 cm	10-20 cm			
District (D)									
Malda	15.34a	12.13a	9.93a	283.98a	197.18a	140.75a			
Coochbehar	12.74b	11.54b	9.29b	218.65b	183.66b	139.95a			
Cropping syster	n (CS)								
RM	14.81a	12.39a	10.56a	274.88a	196.93a	162.81a			
RW	13.65b	11.36b	8.76b	237.07b	185.85b	118.00b			
Tillage (T)	Tillage (T)								
СТ	13.13b	11.32b	10.26a	228.76b	182.08b	151.52a			
ZT	15.33a	12.43a	9.06b	283.20a	200.69a	129.29b			
Analysis of varia	ance (probabil	ity of significa	nce)						
D	<0.001**	0.0654	0.025*	<0.001**	0.051	0.914			
CS	<0.005**	0.002**	<0.001**	0.004**	0.104	<0.001**			
Т	<0.001**	0.008*	<0.001**	<0.001**	0.007	0.004**			
CSxT	0.194	0.563	0.390	0.124	0.990	0.595			
DxCS	0.172	0.928	0.373	0.004**	0.232	0.898			
DxT	0.058	0.644	0.013*	0.295	0.824	0.029*			
DxCSxT	0.374	0.391	0.002**	0.890	0.742	0.398			

Within a column means followed by the same letter are not significantly different (p=0.05) using Tukey's HST; \*shows significance at (p=0.01), \*\*shows significance at (p=0.05); RM= rice-maize system; RW= rice-wheat system; CT= conventional tillage; ZT= zero tillage

Among the field sites (data presented in supplementary table S2), FS4 of Malda recorded highest TOC concentration throughout the depths (0-5, 5-10 and 10-20 cm respectively) as compared to other sites. The least amount of TOC values was recorded in FS6 of Coochbehar. However, the average TOC concentration was approximately 20% higher in the Malda than the Coochbehar soils. The soils under RM system showed significantly (p<0.05) greater amount of TOC concentration which were 8.49, 9.06 and 13.2 % higher than RW system at 0-5, 5-10 and 10-20 cm respectively. With respect to tillage, ZT practice significantly improved the TOC concentration at upper two soil depths (0-5 and 5-10 cm)

respectively which were 16.8 and 9.8 % greater than CT system (Table 2). However, at lower depth (10-20 cm), comparatively higher amount of TOC concentration was found in the soil under CT system (13.24 % higher) in comparison to ZT system.

Depth wise TOC concentration was found to gradually decrease with the increase in depths in soils in general; but the critical perusal of the Table 2 reveal that the depth distribution of TOC concentration differed in proportion when compared among the two districts, cropping systems and the tillage treatments. The concentration of the TOC was found to be more (26%) in 0-5 cm in comparison to 5-10 cm depth in Malda whereas in Coochbehar the same was found to be only 10% higher. Similar comparison of the subsequent depth distribution between 5-10 and 10-20 cm reveal that more or less uniform difference in TOC concentration between the two layers existed in both Malda (22%) and Coocbehar (24%) districts (Figure 1). The trend of the distribution of TOC in the three layers were same in the both the cropping systems with only difference in concentration in each layers was found, where higher quantity of TOC was recorded in RM than the RW system (Figure 1). The comparison of the TOC distribution pattern due to the tillage treatment indicate that under ZT the concentration of TOC was more stratified in 0-5 cm than the other soil layers in comparison to the CT (Figure 1). This is further corroborated from the significant ( $P \le 0.05$ ) interactions of district x tillage (D x T) and district x cropping system x tillage (D x CS x T) for TOC only at 10-20 cm depth (Figure 2 & 3 respectively). The CT practice in Malda soils significantly enhanced the TOC concentration about 20.0 % higher over ZT plot at 10-20 cm depth (Figure 2). Similar result was also observed in Coochbehar soils where CT improved the TOC (4.2%). While, three-way interaction of D x CS x T at 10-20 cm depth showed that practice of CT system under RM significantly enhanced the TOC concentration in Malda district (Figure 3) and with respect to RW system, CT increased the TOC in both the districts.

Among the two labile pools of organic C (POC and HWEC), a significant (p<0.05) increment in HWEC concentration was noticed in Malda (284 mg kg $^{-1}$ ) over Coochbehar (219 mg kg $^{-1}$ ) at 0 $^{-1}$ 5 cm depth but in the subsequent depths, there was no significant difference between the districts was observed in HWEC (Table 2). According the HWEC classes for sandy and loamy soils given by Körschens and Schulz (1999), the HWEC content of FS-1(Malda) and FS-6 (Coochbehar) is low ( $^{-1}$ 200 mg kg $^{-1}$ 1) and greater than the 400 mgkg $^{-1}$ 1 HWEC indicate high concentration was recorded in FS-4 (data presented in supplementary table S2). Practice of RM system significantly ( $^{-1}$ 200 mg kg $^{-1}$ 3 increased the HWEC to the tune of 15.9, 5.9 and 37.9 % at 0 $^{-1}$ 5 and 10 $^{-1}$ 20 cm depths respectively compared to RW. The significant interaction between the district and cropping system at 0 $^{-1}$ 5 cm depth (Figure 2) indicated that the concentration of HWEC was 25% higher for RM than RW system in Malda. While, in Coochbehar, variation was relatively similar between the cropping systems.

While ZT system improved the HWEC (23.7 %) over CT at 0-5 cm but at lower soil depth (10-20 cm) CT increased the same by 17%. The interplay of D x T showed maximum change at lowermost depth (10-20 cm) (Figure-2). CT system in both the districts improved the HWEC at 10-20 cm but in Coochbehar, the HWEC concentration between CT and ZT failed to attain significant difference. The contribution of HWEC fraction to TOC was recorded to be 1.5 to 2.4 % (Figure 4).

Field site and cropping system, significantly affected the POC concentration in both the districts at all depths, while tillage had influenced the POC concentration at selected soil depths (Table 3). Unlike other fractions (TOC and HWEC) which showed maximum concentrations in the Malda soils, the concentration of POC was significantly higher in the Coochbehar soils (Table 3). Significantly (P $\leq$ 0.05) maximum amount of POC (3.61 g kg $^{-1}$ ) recorded in Coochbehar which was 14.2 % higher than Malda (3.16 g kg $^{-1}$ ) at 0–5 cm depth. In the subsequent lower soil depths, the difference of concentration of POC failed to attain significance. Among the field sites studied, FS5 of Coochbehar showed higher POC values throughout the depths (5.04, 3.49 and 3.03 g kg $^{-1}$ at 0–5, 5–10 and 10–20 cm depths respectively) (data presented in supplementary table S2). However, the least amount of POC noted in FS6 of Coochbehar.

Table 3
Effect of environment (District), cropping systems and tillage on particulate organic C (POC) concentration, and mineral associated organic C (MAOC) concentration at different soil depths.

	POC concentration (g kg <sup>-1</sup> )			MAOC cond	MAOC concentration (g kg <sup>-1</sup> )		
	0-5 cm	5-10 cm	10-20 cm	0-5 cm	5-10 cm	10-20 cm	
District (D)							
Malda	3.20b	2.87b	2.45b	12.17a	9.25a	7.48a	
Coochbehar	3.61a	3.03a	2.60a	9.13b	8.50b	6.68b	
Cropping system	m (CS)						
RM	3.73a	3.26a	2.75a	11.08a	9.13a	7.80a	
RW	2.99b	2.62b	2.28b	10.67b	8.74b	6.48b	
Tillage (T)							
СТ	3.15b	2.97a	2.77a	9.99b	8.35b	7.49a	
ZT	3.57a	2.91a	2.27b	11.76a	9.52a	6.80b	
Analysis of vari	ance (probabil	ity of significa	ance)				
D	0.008**	0.327	0.146	<0.001**	0.025*	0.007**	
CS	<0.001**	0.001**	<0.001**	0.173	0.232	<0.001**	
Т	0.009**	0.678	<0.001**	<0.001**	0.006**	0.018*	
CSxT	0.008**	0.019*	0.003**	0.953	0.548	0.056	
DxCS	0.082	0.148	0.046*	0.020	0.428	0.114	
DxT	0.052	0.467	0.145	0.003	0.924	0.058	
DxCSxT	0.375	0.196	0.838	0.642	0.147	0.003	

Within a column means followed by the same letter are not significantly different (p=0.05) using Tukey's HST test; \*shows significance at (p=0.01), \*\*shows significance at (p=0.05); RM= rice-maize system; RW= rice-wheat system; CT= conventional tillage; ZT= zero tillage

A significant (P<0.05) increase in POC recorded under RM system among all depths (3.73, 3.26 and 2.75 g kg $^{-1}$ at 0 $^{-5}$ , 5 $^{-10}$  and 10 $^{-20}$  cm depths respectively) as compared to RW (Table 3). Implementation of CASI under ZT management significantly (P<0.05) enhanced the POC at 0 $^{-5}$  cm depth and CT improved the same at 10 $^{-20}$  cm; while in 5 $^{-10}$  cm soil depth, there was no significant variation observed between the two. However, the interaction effect of CS x T indicated that adoption of ZT under RM system improved the POC at surface depths (0 $^{-5}$  and 5 $^{-10}$  cm) but at the lower depth (10 $^{-20}$  cm), CT showed higher increments. Tillage systems did not affect POC under RW system when referred to 0 $^{-5}$  cm depth,

however, in the lower depths CT improved the POC over ZT (Figure 5). D x CS showed that there was a significant improvement in POC under RM system in Coochbehar compared to RW, but there was a very less difference in concentration noticed between the CS in Malda when referred to 10-20 cm soil depth (Figure 5). The contribution of POC to TOC varied from 18 to 32% in both the Coochbehar and Malda soils (Figure 4).

Concentration of MAOC was found to follow the same trend as that of TOC. The MAOC concentration was more in the soils of Malda (12.2, 9.25 and 7.48 g kg<sup>-1</sup>at 0-5, 5-10 and 10-20 cm depths respectively) which were 33.4, 8.58 and 12.1 % higher than Coochbehar soils (Table 3). FS4 of Malda showed a greater value of MAOC at all the three depths as compared to the other sites (data presented in supplementary table S2). Further, FS2 and FS3 of Malda and FS5 and FS7 of Coochbehar district resulted comparatively similar values of MAOC throughout the depths studied. Effect of cropping system on MAOC observed to be non-significant at surface soil depths, however at 10-20 cm, RM system significantly enhanced the MAOC (7.80 g kg<sup>-1</sup>) over RW (6.48g kg<sup>-1</sup>). Tillage effect on MAOC found to be statistically significant at all the depths. As noticed in TOC, this fraction also improved in the topmost layers by ZT but at the lower depth, CT showed higher values. The interplay of D x CS (Figure 2) and D x T (Figure 5) on MAOC observed to be significant at 0-5 cm depth. Interestingly, RW system enhanced the MAOC in Coochbehar but in Malda, it was more so under RM system when referred to the depth 0-5 cm (Figure 2). With respect to tillage, adoption of ZT significantly increased the MAOC in both the districts as compared to CT at0-5 cm (Figure 5). The three-way interaction of D x CS x Ton MAOC was noticed to be significant at 10-20 cm depth (Figure 3). Practice of CT under RM system in Malda significantly improved the MAOC but not in Coochbehar where ZT system enhanced the same under RM at 10-20 cm. Similarly, CT in Coochbehar and ZT in Malda increased the MAOC under RW when referred to the depth 10−20 cm (Figure 3). The contribution of MAOC to TOC varied from 65 to 80% (Figure 4).

#### Effect of cropping system and tillage on soil BD at different soil depths

In both the districts, soil BD values were increased with the depth (Table 4). However, the higher BD values recorded in Malda (1.33, 1.38 and 1.42 g  $cc^{-1}$  at 0–5, 5–10 and 10–20 cm depths respectively). Among the sites (data presented in supplementary table S2), FS4 of Malda showed maximum BD values; while the minimum values were recorded in FS5 of Coochbehar district. Combined district analysis of the data showed that only the effect of tillage was observed to be significant on soil BD but cropping system failed to show any such variation on BD. CT system significantly decreased the BD over ZT (Table 4) at all the depths. Neither of any interactions recorded significant on BD. The interplay of CS x T observed to be significant (p<0.05) at 0–5 cm depth. Compared to CT, the ZT system enhanced the BD in both RM and RW; however, the difference between CS was negligible (Figure 5). Interaction of D x CS x T presented significant effect on BD (p<0.05) at 10–20 cm depth. Adoption of ZT irrespective of cropping system significantly increased the BD (Figure 3) in both the districts.

Table 4
Effect of environment (District), cropping systems and tillage on soil bulk density and total organic C (TOC) stock at different soil depths.

	Bulk Density (g cc <sup>-1</sup> )			TOC Stoc	TOC Stock (t ha <sup>-1</sup> )			
System	0-5 cm	5-10 cm	10-20 cm	0-5 cm	5-10 cm	10-20 cm		
District (D)								
Malda	1.33a	1.38a	1.42a	10.26a	8.39a	14.12a		
Coochbehar	1.07b	1.14b	1.22b	6.84b	6.60b	11.37b		
Cropping syster	n (CS)							
RM	1.21b	1.28a	1.34a	9.10a	7.93a	14.16a		
RW	1.23a	1.27a	1.33a	8.49b	7.32b	11.72b		
Tillage (T)								
СТ	1.20b	1.27b	1.32b	8.03b	7.20b	13.61a		
ZT	1.24a	1.29a	1.35a	9.55a	8.05a	12.27b		
Analysis of varia	ance (probabi	ility of signific	ance)					
D	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
CS	0.057	0.540	0.256	0.006	0.006	<0.001		
Т	0.004	0.004	<0.001	<0.001	0.003	0.005		
CS*T	0.023	0.222	0.591	0.098	0.760	0.211		
D*CS	0.194	0.873	0.915	0.056	0.653	0.202		
D*T	0.298	0.583	0.808	0.243	0.869	0.006		
D*CS*T	0.602	0.671	0.012	0.746	0.355	0.005		

Within a column means followed by the same letter are not significantly different (p=0.05) using Tukey's HST; \*shows significance at (p=0.01), \*\*shows significance at (p=0.05; RM= rice-maize system; RW= rice-wheat system; CT= conventional tillage; ZT= zero tillage

### Effect of cropping system and tillage on the stock of total organic C (TOC)

TOC stock showed increasing trends with the soil depths (Table 4). At 0–5 cm, TOC stock varied from 8.38 to 13.3 t ha<sup>-1</sup> in the Malda soils and 4.49 to 8.18 t ha<sup>-1</sup> in the Coochbehar soils. Higher stock values recorded in FS4 of Malda and in Coochbehar it was maximum in FS7. The average stock value of TOC in soil was approximately 30% higher in the Malda than the Coochbehar soils. As observed in TOC concentration, RM system showed significantly higher TOC stock as compared to RW at all the depths.

The TOC stocks were significantly higher under the ZT than CT at 0-5 and 5-10 cm; while at 10-20 cm depths it was maximum in CT soils (Table 4). Combined district analysis to study the interaction effect on TOC stock showed that interplay of D x T and D x CS x T observed to be significant only at 10-20 cm depth (Figure 6). CT system substantially enhanced the TOC stock in Malda as compared to ZT but in Coochbehar there were relatively similar TOC stocks recoded under both tillage systems. The soils under CT, in RW system had higher TOC stock at 10-20 cm depth in both the districts. However, CT under RM system in Malda showed significantly (p<0.05) higher TOC stock but in Coochbehar, ZT increased the same (Figure 6).

### Effect of tillage on stratification ratio of TOC, HWEC, POC, and MAOC

The stratification ratio (SR) of TOC, HWEC and POC increased significantly with increased soil depth, and they differed significantly among tillage treatments at all the sites of both Coochbehar and Malda (p<0.05, Table 5). Stratification values of TOC, HWEC and POC at 0–10 cm (0–5/5–10: d1/d2) and0–20 cm (0–5/10–20: d1/d3) was significantly higher in the ZT as compared with CT. The SR of d1/d3 showed maximum values of TOC stratification against d1/d2. Following the same trend as that of TOC, stratification of HWEC recorded higher ratio values in d1/d3 as compared with d1/d2 and higher under the ZT than CT (Table 5) except in the FS-1 and FS-4 with respect to d1/d2. Similarly, strtaification of POC (Table 5), recorded higher ratio values in d1/d3 compared to d1/d2 indicating less concentration at the lower depths (10–20 cm) in ZT. Only, one exception was recorded in FS-5 where SR of POC was recorded to be higher in CT for both the d1/d2 and d1/d3. The Malda soils showed higher stratification values of TOC, HWEC and POC in comparison with Coochbehar soils under ZT management practice (Table 5).

Table 5
Effect of tillage systems on the stratification ratios of TOC, HWEC and POC

Distrits	Field site	Depth ratio	Tillage	TOC	HWEC	POC
	(FS)					
Malda	FS-1	d1/d2	СТ	1.25	1.19	0.89
			ZT	1.22	1.13	1.17
			<i>p</i> value	NS	NS	0.03*
		d1/d3	CT	1.66	1.65	1.12
			ZT	1.81	2.03	1.55
			<i>p</i> value	NS	0.06*	0.04*
	FS-2	d1/d2	СТ	1.17	1.15	0.88
			ZT	1.35	1.68	1.11
			<i>p</i> value	0.01**	0.01**	NS
		d1/d3	СТ	1.22	1.41	0.96
			ZT	1.69	2.35	1.13
			<i>p</i> value	0.01**	0.01**	NS
	FS-3	d1/d2	СТ	1.33	1.98	1
			ZT	1.48	2.29	1.41
			<i>p</i> value	0.05*	0.04*	0.02*
		d1/d3	СТ	1.32	1.96	0.97
			ZT	2.18	3.08	2.15
			<i>p</i> value	0.01**	0.01**	0.01**
	FS-4	d1/d2	СТ	1.24	1.31	1.04
			ZT	1.15	1.26	1.37
			<i>p</i> value	0.04*	NS	NS
		d1/d3	СТ	1.29	1.58	1.02
			ZT	1.62	2.54	2.04

<sup>\*</sup>  $d_1/d_2$ :0-5/5-10;  $d_1/d_3$ :0-5/10-20 and p value shows significant at <0.05 level. TOC = total organic carbon; HWEC = hot water extractable carbon; POC = particulate organic carbon; CT = conventional tillage; ZT = zero tillage; NS-non significant, \*shows significance at (p=0.01), \*\*shows significance at (p=0.05); FS= field site

Distrits	Field site	Depth ratio	Tillage	TOC	HWEC	POC
	(FS)					
			<i>p</i> value	0.03*	<0.01**	<0.01**
Coochbehar	FS-5	d1/d2	СТ	1.07	1.13	1.75
			ZT	1.12	1.29	1.21
			<i>p</i> value	NS	0.01**	0.01**
		d1/d3	СТ	1.4	1.75	1.71
			ZT	1.73	2.2	1.62
			<i>p</i> value	0.01**	0.01**	NS
	FS-6	d1/d2	СТ	0.88	0.87	0.85
			ZT	1.2	1.23	1.15
			<i>p</i> value	<0.01**	0.04*	0.04*
		d1/d3	CT	0.87	0.94	1.11
			ZT	1.45	1.44	1.32
			<i>p</i> value	<0.01**	0.05*	NS
	FS-7	d1/d2	CT	1.09	1.11	0.94
			ZT	1.2	1.35	1.2
			<i>p</i> value	NS	NS	NS
		d1/d3	CT	1.2	1.1	1
			ZT	1.48	1.72	1.46
			<i>p</i> value	<0.01**	0.03*	0.01**

<sup>\*</sup>  $d_1/d_2$ :0-5/5-10;  $d_1/d_3$ :0-5/10-20 and p value shows significant at <0.05 level. TOC = total organic carbon; HWEC = hot water extractable carbon; POC = particulate organic carbon; CT = conventional tillage; ZT = zero tillage; NS-non significant, \*shows significance at (p=0.01), \*\*shows significance at (p=0.05); FS= field site

# Relationship of total organic C (TOC) with hot water extractable C(HWEC), particulate organic C (POC) and mineral associated C (MAOC)

Considering all the depths, a strong positive correlation (r= >0.80,  $p \le 0.01$ ) was observed between TOC and HWEC (Table 6A), both in the Malda and Coochbehar soils. Interestingly, we observed that POC concentration was influenced by the soil texture differently in the soils of Coochbehar and Malda. In the

Malda soils, there was a negative correlation (Table 6B) between POC and sand (r=-0.59\*, -0.54\*, and -0.43\*, p<0.05), but these were positively correlated in the Coochbehar soils (r=0.73\*\*, 0.78\*\* and 0.85\*\*,) at 0-5, 5-10 and 10-20 cm depths, respectively. It was also observed that silt was positively correlated with POC concentration in the Malda soils, but negatively correlated in the Coochbehar soils. Relationship of TOC with texture showed that in the Malda soils it was highly correlated with clay content (r=0.78\*\*) but in the Coochbehar soils, it was strongly correlated with sand content (r=0.53\*) (Table 6C).

### **Discussion**

In this short-term (4 year) study adoption of CASI practice under ZT management significantly influenced the concentration of soil C fractions, TOC stock and its depth-wise distribution compared to CT. The variability of TOC concentration in different sites of Coochbehar and Malda was observed is due to the background TOC content and difference in crop management practices adopted by the different farmers. This is further corroborated from the statistical study which indicate that the effect of district and villages (sites) were significant.

TOC concentration was found to be significantly (*p*<0.05) higher in the RM cropping system compared to RW. Since the addition of C substrate is more in the RM (10 Mg ha<sup>-1</sup>) than RW (5.5 Mg ha<sup>-1</sup>) (Sinha et al 2019) due to higher biomass of maize crop, this will naturally increase the TOC concentrations as well as the C stocks (Jat et al., 2019). Several long-term studies reported that the concentration of labile C pools depends on the amount of substrate input (Cambardella and Elliott, 1992; Janzen et al., 1992; Choudhary et al., 2018). Addition of more crop residues to the soil increases the OC status of soil (Somasundaram et al., 2018). TOC and labile C fractions showed higher levels in the treatments, where both rice and wheat straw were retained compared to only wheat or rice straw return, indicating the importance of straw return in improving the soil TOC and its fractions (Zhu et al., 2014; Jat et el., 2019). Concentration of C fractions depends largely on the amount of organic residues added to the soil (Somasundaram et al., 2018).

The ZT practice significantly enhanced the TOC and its fractions over CT practice. Similar higher TOC concentration in the ZT system was also reported by Alvarez et al. (1995) and Metay et al. (2007). Concentration of TOC and its fractions under the ZT were maximum up to 10 cm soil depth, but in the subsequent depth, the ZT failed to show any significant (p<0.05) improvement compared to CT practice, where TOC and its fraction, recorded higher values at10–20 cm depth. Similar higher levels of TOC under the ZT than CT at 0–10 cm also reported by Angers and Eriksen-Hamel (2008). The ZT with wheat residues retained practice significantly enhanced the TOC concentration at 0–15 cm depth over CT in some soils (Chen et al., 2009; Rajan et al., 2012; Hati et al., 2015).

The stocks of TOC were observed to be increasing with depth under the effect of different tillage practices (ZT and CT) with two cropping systems (RW and RM) among all the sites studied in the Malda and the Coochbehar soils. Cropping systems under the CT practice showed higher TOC stocks at lower soil depths (below 10 cm) due to the mixing and inversing i of residues during tillage (Kumari et al., 2011; Jat et al., 2019). Dimassi et al., (2013) reported that the TOC stocks below the old plough layer (28–40

cm) were slightly greater in full inversion tillage than in no-tillage treatment. A similar trend of the soil under CT having higher TOC concentration at the lower depth (10–20 cm) by 18 % than ZT, was also reported by Zhu et al. (2014). Although there was no residue incorporation in the ZT, the higher TOC stocks were recorded at lower soil depths, which may be ascribed to the similar soil textural characteristics (low clay and high silt content) in the soils at these three sites (FS-1: 16 and 66 %; FS-6: 10 and 62 %; FS-7: 12 and 60 %, respectively) (Table 1). This may have enhanced the movement of organic carbon fractions which constitute and are the component of the TOC, into the lower layers. Implementation of ZT in the Malda soils, improved the concentration of TOC fractions at the upper depths as the soils were rich in clay content (28%) (Table 1).

Therefore, in this study there is relatively higher TOC concentration in the surface (0-5 cm) in comparison to the subsequent depths (5-10 and 10-20 cm). Depth-wise distribution of TOC fractions decreased with increasing depth. Somasundaram et al., 2018 also observed a marked decrease in the very labile C fraction with increasing soil depth. However, the TOC stocks increased with depth reflecting higher soil bulk density. The variation in the pattern of the distribution of the TOC and its stock was influenced by both the textural differences and the tillage treatments which influenced the distribution of the residue within and on the soil. Irrespective of the site characteristics, the RM system added more C input than the RW system in the soil resulting in higher TOC concentration in all the three soil depths.

We found a significant positive relationship between clay and TOC in the Malda soils but a positive correlation with the sand and negative correlation with silt in the Coochbehar soils (Table 7C). This is a peculiar phenomenon and is the characteristics of the Coochbehar soils, which are low in clay content. Therefore, the role of clay may vary by region (Oades, 1988; Goncalves et al., 2017). Hassink (1997) attributed this relationship due to the formation of organo-mineral complexes between the organic matter particles and the clay forming bond which stabilizes the C in the soil. The process and the extent of binding always varies among the different clay types (Blanco-Canqui and Lal, 2004). The clay content and mineralogy also regulate the TOC pools by influencing the sensitivity of soil C to microbial attack (Percival et al., 2000; Kumari et al., 2011; Choudhary et al., 2018), therefore, we expect higher rate of TOC turnover in the Coochbehar soils (recent alluvial Entisols) than the Malda (old alluvial Inceptisol) soils. Moreover, the moisture content in the soils of Coochbehar is relatively higher than that in the Malda soils, which allows the labile pools to move down the soil profile with the water movement.

A strong and positive correlation of HWEC with TOC (*r*=0.76, n=84) (Figure 7) showed that the concentration and the distribution of HWEC is directly influenced by the organic C concentration of the soil. Similar relationship between TOC and HWEC was also reported elsewhere (Spohn and Giani 2011; Haney et al., 2012;Vladimiret al.,2016). This labile form of carbon is also related to the microbial biomass (Sparling et al., 1998) and micro-aggregation (Puget et al., 1999) in soil and is therefore an important indicator of soil quality (Ghani et al., 2003). The concentration HWEC of the two sites (FS-1 and FS-4), according to the classes given by Körschens and Schulz (1999) is highly depleted in organic carbon (<200 mg kg<sup>-1</sup>). This variation in the concentration at different sites under is due to the background TOC content of the soils of these regions. Intervention with respect to the tillage and cropping system in the

long run would improve the concentration in soil and also its quality. Depth-wise distribution of HWEC followed a similar trend as that of TOC; while an increased level was observed in the 10–20 cm soil depth under CT practice over the ZT practice; this was certainly due to the incorporation of residues in these treatments. He et al., (2009) observed a significant difference between tillage systems at soil depths down to 20 cm, but not deeper in the soil profile.

Concentration of POC was significantly higher under the RM system than RW; this was ascribed to the fact that the C input rates are basically higher in the RM system over the RW, which substantially increased the concentration of POC. After 6 years of the experimentation, Jat et al. (2019) also reported that the maize-wheat system had higher TOC and POC than rice-wheat system under no-till condition; this was attributed due to 10 t ha<sup>-1</sup> more biomass added in the fomer than the latter system. Addition of higher biomass increased the level of POC, which was also reported by Mapfumo et al., (2007). The POC concentration in the soils was comparably higher at 0–5 cm under the ZT than CT; while at 10–20 cm depths, the concentrations were higher under CT irrespective of cropping systems. Different quantity of residues retained between the CT and ZT also affect the amount of POC at different depths. Elsewhere also, it was observed that the POC was greater under ZT than under CT at 0–6 cm depth but was lower under ZT than under CT at 6–30 cm (Franzluebbers and Stuedemann, 2014; Zhongming et al., 2016.

Interestingly, our study showed that the POC concentration was strongly influenced by the texture of the soils (specifically sand) at 0-20 cm depth. We observed a negative correlation (Table 7B) between POC concentration and sand in the Malda (Inceptisol) soils and a positive correlation between them in the Coochbehar (Entisol) soils at all the three depths. It has been reported (Six et al. 2002 and Nciizah & Wakindiki 2012) that the fresh POM is sequestered by the sand particles whereas clay physically and chemically protect the decomposed POM in soils. Therefore, a negative correlation of silt and clay fraction with POC concentration in the Coochbehar soils indicated that the strong association or stabilization of C may not occur in the Coochbehar due to the lower amount of clay (11.7%) (Table-1). However, in the Malda soils, a significant proportion of POC such as POC occluded within aggregates may be much more protected due to higher clay and silt content of the soil, as it showed a positive relationship with these soil properties. Six et al. (2002) and Kumari et al. (2011) defined the unprotected POM as the 53-2000 µm size, not contained within micro-aggregates and protected POM as 53-250 µm sized contained within micro-aggregates. Therefore, the correlation data indicates, there may be a difference in POC distribution in different size fractions (unprotected and protected) in the Coochbehar and Malda soils. It also points towards the fact that in Coochbehar a portion of SOM is partially decomposed and more recent in origin than the SOM of Malda soils. The poor correlation (Figure 7) of POC with TOC (r=0.27, n=84) further corroborates the fact that the POC/POM is more linked to the residue and their decomposition state rather than the soil characteristics. As we have seen in the case of the Coochbehar soils, the TOC concentration of the selected sites was lower than the Malda soils although the former had a higher POC concentration. Many studies have reported diverse results on the effects of conservation agriculture on POC (Somasundaram et al., 2017).

Concentrations of MAOC at 0–5 cm depth was maximum under the ZT practice, due to residues remained at the soil surface as compared to the CT practice, where the residues were incorporated in the tilled layer (0–20cm). These results corroborate the findings of Somasundaram et al. (2017). A strong relationship between MAOC and TOC found in our study (Figure 7) indicated a favourable increment of MAOC with TOC. The MAOC is formed upon binding of organic matter (OM) to clay and silt particles (Mikutta and Kaiser 2011). Plant-derived labile compounds act as a main source of C binding agent to the mineral fraction (Cotrufo et al.,2015). Such labile compounds bind to the mineral fractions or incorporated into microbial biomass (Castellano et al., 2015). Promotion of soil C accumulation under ZT occurs from organic C input from residue retention. This is also observed in other studies. A significant contribution of crop residues to TOC at 0–10 cm occurred in the silt and clay fractions, which indicated that most of the young TOC was protected in the form of MAOC fraction under a long (10-year) no-tillage study (Saet al., 2001).

In the present study, the stratification ratio (SR) of TOC, HWEC, and POC progressively increased with increase in the soil depth, due to the decrease in the TOC concentrations along the soil depth (Table 6). Compared with CT, the stratification of TOC with depth is a spontaneous process which is mainly induced by the continuously higher input of C at the soil surface and less in the subsequent soil layers under ZT treatment, irrespective of the site and the cropping system. Similar results were reported by Sa and Lal (2009) and Ferreira et al. (2013). Franzluebbers (2002) reported that stratification ratios of soil C and N pools for the four soils in Alberta and British Columbia were minimally, and variably affected by tillage system, which was unlike that observed for soils in Texas and Georgia. He observed that there was high stratification ratio of soil C and N pools under CT in Alberta/British Columbia, clearly indicated that the soil degradation with inversion tillage may not have been affected in comparison to the other regions. He also considered other factors and among them variability in climate, played a significant role in Alberta. In the context of present study, similar variation in TOC, HWEC, and POC was due to variable mixing of the residues in CT at the three sites (FS-1,4 and 5), which resulted in comparatively lower amount of TOC, HWEC and POC at 5-10 cm than ZT. Similarly, in FS-5, the SR was found to be higher in CT than ZT in 0-5/10-20 cm (Table 6). Melero et al. (2012), also observed that the relative proportion of variation within the factors contributing to the variations (tillage 54±15%, soil depth increment 25±14%, crop rotation 13±7%, and N fertilizer rate 8±3%) in the SR of TOC. The results presented in our study, indicate that the SR of the Malda soils was higher for TOC, HWEC and POC under the ZT than that under CT (Table 6), due to the inherent higher stock of TOC and the heavier soil texture in the soils of old alluvial Inceptisol (Malda) than the recent alluvial Entisol (Coochbehar) (Table 5).

Table 6
Correlation matrix between different parameters

A. Relation between HWEC and TOC									
Soil depth (cm)	Malda			Coochbe	Coochbehar				
0-5	0.935**			0.940**					
5-10	0.898**			0.867**					
10-20	0.896**			0.920**					
B. Relation betwe	B. Relation between POC and soil texture								
Soil depth (cm)	Malda			Coochbehar					
	Sand	Silt	Clay	Sand	Silt	Clay			
0-5	-0.585*	0.496	0.425	0.725**	-0.797**	-0.189			
5-10	-0.536*	0.498*	0.367	0.781**	-0.746**	-0.43			
10-20	-0.426	0.476	0.38	0.854**	-0.676**	-0.048			
C. Relation betwe	een TOC an	d soil text	ure						
	Sand		Silt		Clay				
Malda	-0.263		-0.034		0.783**				
Coochbehar	0.529*		-0.660*	*	0.121				

*Note:* \* and \*\* represent that correlation is significant at the 0.05 and 0.01 level (2-tailed) respectively; HWEC = hot water extractable C; TOC = total organic C; POC = particulate organic C

The overall scenario with respect to the status and distribution of TOC and its fractions, in the seven sites was found to vary due to the tillage, cropping system, and soil types. From the interaction effect (CS  $\times$  T), our results showed that the RM system in CASI under ZT improved the TOC and its fractions over CT compared to RW. The ZT practice enhanced the concentration of TOC by 18.1% over CT under a long-term maize cropping system (Huang et al. 2010). Retention of the residue on the surface under ZT, generally reduces contact with the soil and fluctuations in soil moisture and temperature at surface depth reduces the decomposition rates. Compared to residues left on the soil surface, incorporation in CT results in decomposition at a 3–4 times higher rate (Beare et al., 1993; Choudhary et al., 2018; Jat et al., 2019). The slow decomposition of the residue on the surface results in a slower rate of incorporation of these residues into the soil, and increases the amount of SOC in surface soil depth (Ghimire et al., 2012; Jat et al., 2019). Consequently, stratification ratios varied accordingly and strongly influenced by the soil texture. In the Coochbehar soils (sandy loam textured, Entisols), movement of TOC and its fractions may have occurred into the soil profile, resulting in lower stratification than that in the Malda soils of finer soil textured Inceptisol.

### **Conclusions**

At the end of seven cropping seasons, the study showed a strong correlation of C fractions with TOC under both the CT and ZT practices and the RM and RW cropping systems. The result indicates that the labile C fractions represent a portion of TOC with different turnover rates and are important in judging the soil quality. The CASI practice under ZT increases the soil sequestration of C due to the addition of residue in the soil, though this increase varied with cropping system while the RM proved superior to RW. The RM system increased the C turnover rate in both soil types and the amount of clay in these soils influenced the stabilization/storage of C. Placement of residues on the surface results in slow decomposition of the residues and hence gradual loss of the added organic matter helps in reducing the loss to the environment. The concentration of TOC and its fractions increased with increasing soil depths. The contribution of C fractions to TOC were in the order: MAOC (65-80%)>POC (18-32%)>HWEC (1.5-2.4%). The heavier textured Inceptisols could accumulate more C fractions compared to light textured Entisols and the former soils has a strong association or stabilization of C which corroborated from positive correlation (p<0.05) of TOC and POC with clay. Stratification of the C in 0–5 cm soil depth may result in an imbalance in the distribution of C which is more prominent in clayey soils (old alluvial Inceptisol) than the sandy soils (recent alluvial Entisol). The input of organic material is critical to the long-term maintenance of SOM. Therefore, the residue management practices are likely to affect organic matter content in different soil types under different tillage and cropping systems.

### **Abbreviations**

ACIAR = Australian Council for International Agriculture Research

ANOVA = Analysis of variance

BD = Bulk density

CA = Conservation agriculture

CASI = Conservation Agriculture based Sustainable Intensification

CIMMYT = International Maize and Wheat Improvement Center

CS = Cropping system

CT = Conventional tillage

D = District

FS = Field sites

GHG = Green House Gas

GLM = General linear model

HSD = Tukey's honest significance difference

HWEC = Hot water extractable/soluble carbon

IPCC = Intergovernmental Panel on Climate Change

MAOC = Mineral associated organic carbon

OM = organic matter

POC = Particulate organic carbon

PTR = Puddled transplanted rice

RM = Rice-maize system

RW = Rice-wheat system

SOC = Soil organic carbon

SOM = Soil organic matter

SR = Stratification ratio

SRFSI = Sustainable and Resilient Farming Systems Intensification

T = Tillage

TOC = Total organic matter

UBKV = Uttar Banga Krishi Viswavidyalaya

UNEP = United Nations Environment Programme

UPTR = Unpuddled transplanted rice

ZT = Zero tillage

### **Declarations**

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#### **Conflicts of Interest**

The authors declare that they do not have any financial and personal conflict of interest

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# **Figures**

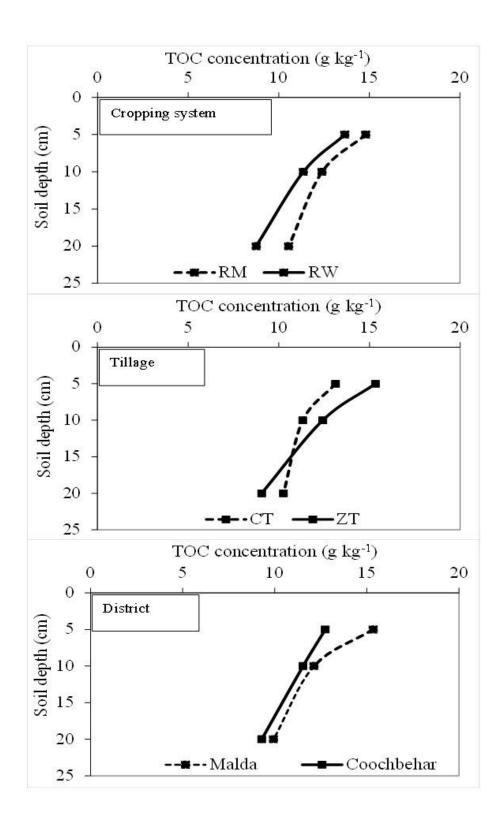


Figure 1

Depth wise (0-5, 5-10 and 10-20 cm) distribution of TOC concentration as affected by the district, cropping systems and tillage practice. CT=conventional tillage; ZT=zero tillage

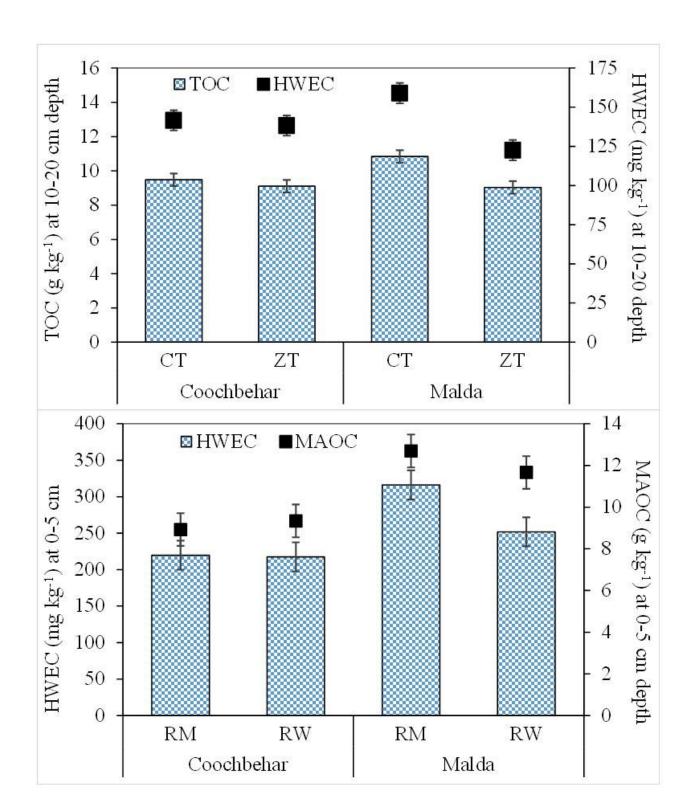


Figure 2

Interactive effect of district, cropping system and tillage practice on different fractions of organic C concentration at different soil depths. TOC=total organic C; HWEC=hot water extractable C; MAOC= mineral associated organic C; CT=conventional system; ZT= zero tillage; RM=rice-maize system; RW=rice-wheat system.

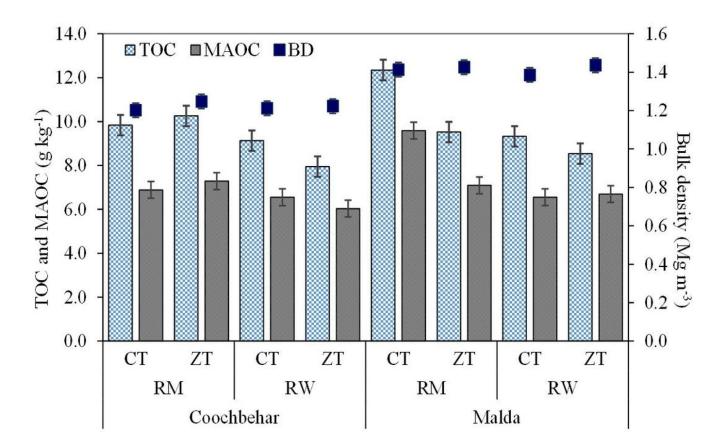
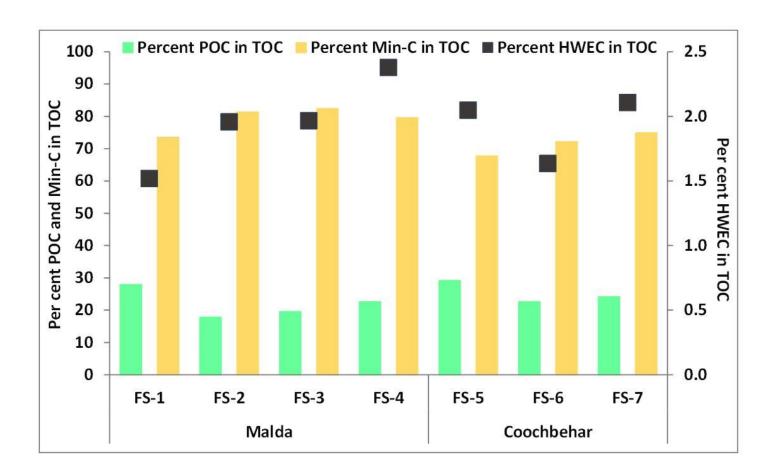


Figure 3

Interactive effect of district, cropping system and tillage practice on different fractions of organic C concentration and BD at 10-20 cm soil depth. TOC=total organic C; MAOC= mineral associated organic C; BD=soil bulk density; CT=conventional system; ZT= zero tillage; RM=rice-maize system; RW=rice-wheat system.



Percent of hot water extractable C (HWEC), particulate organic C (POC) and mineral associated organic C

(MAOC) of total organic C (TOC) varies at different field sites of Malda and Coochbehar.

Figure 4

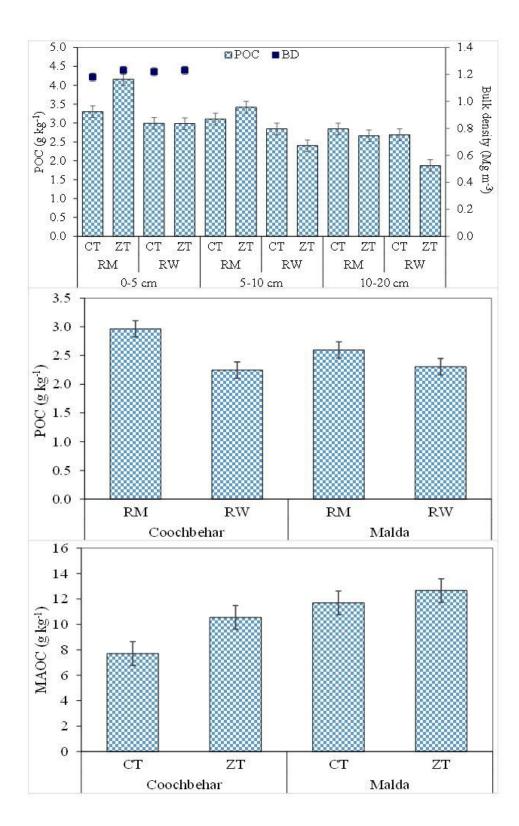


Figure 5

Interactive effect of district, cropping system and tillage practice on different fractions of organic C concentration at different soil depth. POC=particulate organic C; MAOC= mineral associated organic C; CT=conventional system; ZT= zero tillage; RM=rice-maize system; RW=rice-wheat system. Within depth and same color, horizontally different letters are significantly different (p=0.05) using Tukey's HST test. ns=non-significant

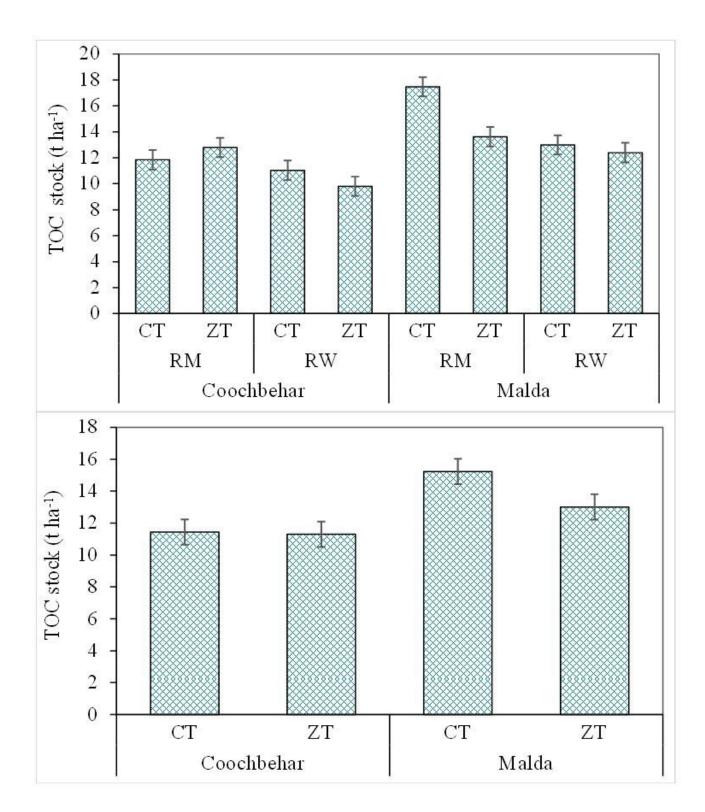


Figure 6

Interactive effect of district, cropping systems and tillage practice on TOC stock at 10-20 cm soil depth. TOC-stock=total organic C stock;CT=conventional system; ZT= zero tillage; RM=rice-maize system; RW=rice-wheat system.

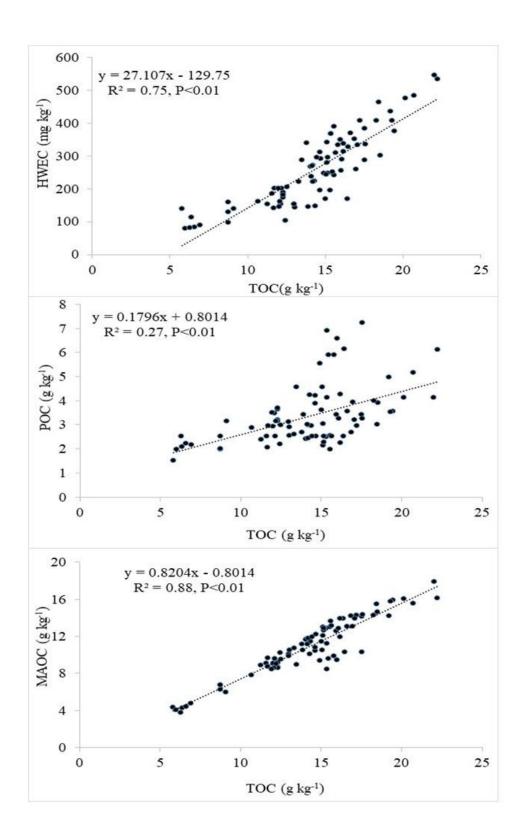


Figure 7

Relationship of total organic C (TOC) with hot water extractable C (HWEC), particulate organic C (POC) and mineral associated organic C (MAOC).

# **Supplementary Files**

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