

Original Research Article

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Effect of Long-Term Application of Inorganic and Organics on Soil Aggregate Fractions in Double Rice Cropping System

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ABSTRACT

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Studying the dynamics of soil organic carbon (SOC) is important for understanding the carbon stabilization into different pools. Thus, a 25-year old experiment was used to assess the impact of double rice cropping system with addition of organics and grades of fertilization on labile carbon pools and crop yield sustainability in an Inceptisol in southern India. The total water stable aggregates (WSA) in the experimental soils ranged from 70.4 to 94.9% under different treatments. Geometric mean diameter (GMD) also exhibited similar trend which ranged from 1.32 to 1.42 mm. Aggregate ratio (AR) showed similar trend, with the highest values in soils under FYM treatment (8.76) but lowest values in soil under control (2.40). Application of organics had immense effect on soil aggregation and C sequestration. Mesoaggregates shared the maximum proportion of aggregate associated C (35.8%) followed by fine micro, coarse micro, coarse macro aggregates and silt + clay fractions constituting 26.2, 14.3, 12.5 and 11.2%, respectively.

Introduction

Analyses of physical structure of soil are usually done using physical fractionation methods, which are based on the premise that the association of the soil particles and their spatial arrangement play a key role in the function of SOM (Gregorich *et al.*, 2006). The aggregation is a means to both protect and conserve soil organic carbon (SOC) and allow the stored organic matter to function as a reservoir of plant nutrients. Crop cultivation is known to adversely affect the distribution and stability of soil aggregates and reduces SOC stock in soils (Six *et al.*, 2000; Kong *et al.*, 2005). The impacts of cultivation on C stock have commonly been observed to be

restricted mostly to surface soils and/or to root zone depth (Paustian *et al.*, 1997b). However, different crop species have different effects on soil aggregation and C accumulation with varying soil depth. Altering soil physicochemical properties by management practices may increase one or more of the protective attributes which ultimately increases C in soils provided C inputs to soil do not decrease.

The distribution of soil organic carbon (SOC) in different aggregate size classes (i.e. micro, meso, and macroaggregates) may affect soil erosion and more rapid loss may occur from

macroaggregates than microaggregates (Eynard *et al.*, 2005). Tillage disturbs large aggregates more than smaller aggregates, making SOC more susceptible to mineralization (Six *et al.*, 1998). Clay particles have a higher protective effect on chemical and biophysical processes of carbon stabilization (Christensen, 1996). Clay plus silt serves as a fixed capacity level (Hassink and Whitmore, 1997) while the combination of micro, meso, and macro aggregated carbon provide an additional variable capacity. The former is soil specific while the latter tends to be contingent on both amount of carbon input and soil type.

Cultivated soils have a smaller WSA within >2 mm and 1-2 mm aggregate size fractions but a greater aggregation in <0.25 mm size fraction than the fallow is found. Tillage operations may enhance the susceptibility of aggregates to disruption by wet-dry cycles that lead to a loss of C-rich macro aggregate fractions. The MWD and GMD have smaller values in the cultivated than the fallow soils indicating more disturbances through tillage and lower accumulation as well as protection of SOC in macro-aggregates (Gupta Choudhury *et al.*, 2010).

The smaller aggregates in the cropland soils are therefore consistent with the lower SOM content (Emadi *et al.*, 2008). Loss of the larger aggregate sizes in cropland could also be due to tillage rapidly destroying live and decaying plant roots, fungal hyphae, earthworms and termites. These factors tend to favor the formation of larger sized aggregates (Tisdale and Oades 1982). Size distribution of aggregates is affected by the change in land use and management (Spaccini *et al.*, 2001). The loss of large sized water stable aggregates under cultivation was also associated with a significant reduction in stability as measured by the MWD. The stability of intact WSA showed higher values in uncultivated soils than in cultivated soils.

There were no significant differences in MWD between forest and pasture soils (Emadi *et al.*, 2008) Although the importance of organic matter to improve soil aggregate stability is well known, the experiments showing the beneficial effects of organic matter on aggregate stability have been varied. For instance, some workers (Christensen 1986) found a significant correlation between organic matter and aggregate stability.

Materials and Methods

Site description

A long-term experiment was established in the year 1989, at the experimental farm of the Andhra Pradesh Rice Research and Regional Agricultural Research Station, Maruteru, Andhra Pradesh (16.37°N and 16.40°N, and 81.44°E and 81.46°E and 8.2 m above mean sea level) with double rice (*Oryza sativa* L.). Crop was sown every year during *kharif* i.e. mid June to mid-October and *rabi* i.e. mid November to mid-March. The area receives, on an average, annual rainfall of approximately 1200 mm. The mean annual minimum and maximum temperatures were 10.0°C and 37.6°C, respectively.

The soil was classified as *inceptisol*, with clay loam texture developed from the deltaic sediments of Godavari River. The site had the soil moisture and temperature regimes of udic and mesic/thermic, respectively.

Two rice (*Oryza sativa* L.) crops (varieties: MTU-2067, 1061, 1010) were grown annually in the experiment. The experiment was laid out in randomized complete block design with three replications and consisted of the following treatments: (i) control (plots without NPK fertilizers and organics), (ii) 50% NPK (45:30:30), (iii) 50% NPK + 50% N- green manure (*GM-Calotropis* sps.), (iv) 50% NPK + 50% N- farmyard manure, (v)

50% NPK + 25% N- green manure (*Calotropis* sps.) + 25% N- farmyard manure, (vi) farmyard manure (10 t ha⁻¹), (vii) 100% NPK + farmyard manure (5 t ha⁻¹), (viii) 100% N and (ix) 100% NPK (90:60:60).

Aggregate analysis and structural indices

Two sets of six nested sieves with 2000, 1000, 500, 250, 100 and 53 µm diameter size class were used for the separation of water stable aggregates and subsequent calculation of different structural indices. Aggregate separation was done by using wet sieving apparatus (Yoder, 1936). After removing visible pieces of crop residues and roots from the field-moist soil samples, aggregates ranging in diameter from 2000 to 5000 µm were obtained from the air-dried bulk soil that had been broken apart by hand before air-drying for the wet sieving procedure. Exactly 100 g of soil aggregates (2000 to 5000 µm) in duplicate was slaked by submerging it in deionized water placing on top 2000 µm sieve for a while at room temperature. Water stable aggregates were then separated by moving the sieves up and down in a Yoder apparatus for 30 minutes. After correcting sand content in all the aggregates by dispersion with sodium hexametaphosphate, soil aggregate indices were calculated. Aggregates were then fractioned into coarse macro aggregates (CMacA, >2000 µm), mesoaggregates (MesoA, 250-2000 µm) and coarse microaggregates (CMicA, 100-250 µm). The sum of aggregates >250 µm was clubbed as macroaggregates (MacA) while aggregates <250 µm grouped into microaggregates (MicA). With the data of soil aggregates and primary particles the following soil aggregate indices were calculated.

Water stable aggregates

From the weight of the soil particles (Aggregates + primary particles) in each size

group, its proportion to the total sample weight was determined. Water stable aggregates (WSA) was the mass of stable aggregates divided by the total aggregate (stable + primary particles) mass as

$$\text{Water stable aggregates (\%)} = \left[\frac{(\text{Weight of soil + sand})_i - (\text{Weight of sand})_i}{\text{Weight of sample}} \right] \quad (1)$$

Where, *i* denotes the size of the sieve. The percentage weight of water stable macroaggregates is the summation of soil aggregate-size fractions > 250 µm; while the percentage weight of water stable microaggregates are those retained in < 250 µm.

Aggregate ratio

Aggregate ratio (AR) is denoted by

$$\text{Aggregate ratio} = \left[\frac{(\text{Aggregates retained in } > 250 \mu\text{m})}{(\text{Aggregates retained in } < 250 \mu\text{m})} \right] \quad (2)$$

Mean weight diameter

After correction of sand content, the amount of aggregates remaining in each size fraction was used to calculate the mean weight diameter (MWD) of the water stable aggregates following van Bavel (1949) as:

$$\text{Mean weight diameter (mm)} = \frac{\sum_{i=1}^n X_i W_i}{\sum_{i=1}^n W_i} \quad (3)$$

Where, *n* is the number of fractions (100-250, 250-500, 500-1000, 1000-2000, > 2000 µm, *X_i* is the mean diameter (µm) of the sieve size class (0.175, 0.375, 0.75, 1.5 and 2.0 mm) and *W_i* is the weight of soil (g) retained on each sieve.

Geometric mean diameter

Geometric mean diameter (GMD) an exponential index of aggregate stability was expressed as:

$$\text{Geometric mean diameter (mm)} = \exp \left[\frac{\sum_{i=1}^n W_i \log X_i}{\sum_{i=1}^n W_i} \right] \quad (4)$$

Where, n is the number of fractions same as MWD size, X_i is the mean diameter (mm) of the sieve size class same as MWD size and W_i is the weight of soil (g) retained on each sieve.

Percent aggregate stability

The index percent aggregate stability or degree of aggregation (AS) of soil was calculated as:

$$\left(\frac{\text{Percent soil particle } > 250 \mu\text{m} - \text{Percent primary particles } > 250 \mu\text{m}}{\text{Percent primary particles } < 250 \mu\text{m}} \right) \quad (5)$$

Readily oxidisable organic carbon (OC)

The oxidizable organic carbon (OC) was determined by Walkley and Black wet oxidation method (Walkley and Black, 1934). One-half g of ground (< 2.0 mm) soil was placed in a 500 ml Erlenmeyer flask to which 10 ml of 1.0 N $K_2Cr_2O_7$ was first added, followed by 20 ml concentrated sulphuric acid.

After half an hour of the reaction under dark, the excess dichromate was determined by titrating against 0.5 N $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. The amount of dichromate consumed by the soil was used to calculate the amount of OC based on the theoretical value of 1.0 ml 1.0 N $K_2Cr_2O_7$ oxidises 3.0 mg C.

Statistical analysis

Means of three replicates and standard errors of the means were calculated for all the pools of soil organic carbon (on dry weight basis). The data were analysed using randomized block design (RBD). Statistical analysis was performed by DOS-based SPSS version 12.0. The SPSS procedure was used for analysis of variance (ANOVA) to determine the statistical significance of treatments as well as of cropping systems. Two factor factorial ANOVA was used to determine the existence of interaction effect between treatments and cropping systems. Simple correlation coefficients and regression equations were also developed to evaluate relationships between the response variables using the same statistical package. The 5.0% probability level is regarded as statistically significant.

Results and Discussion

Water stable aggregates and structural indices

The total water stable aggregates (WSA) in the experimental soils ranged from 70.4 to 94.9% under different treatments. Among the treatments, RDF+FYM had the highest value of different size distributions of water stable aggregates (WSA) (>2000 μm , 2000-1000 μm , 1000-500 μm , 500-250 μm , 250-100 μm) followed by 50% RDF+GM > 50% RDF+FYM > FYM > 50% RDF+GM+FYM > RDF > 50% RDF > N > control (Table 1). The mesoaggregates, MesoA (2000-250 μm) comprised of 2.0 - 5.1% of total WSA compared to 91.6-96.8% as coarse macro aggregates (CMacA, > 2000 μm) and 0.26-2.35% as coarse micro aggregates, (CMicA, 250-100 μm).

Irrespective of the treatments, the mesoaggregates (MesoA, 250-2000 μm)

comprised of 4.2% of the total WSA compared to 94.6% as coarse macro aggregates (C_{MacA}, > 2000µm) and 1.3% as coarse micro aggregates, (C_{MicA}, 250-100 µm). Again, among the mesoaggregates, the 1000-2000 µm fraction exhibited a higher value followed by 500-1000 and 250-500 µm fractions constituting, on an average, 2.1, 1.2 and 0.9% of the total mesoaggregates.

Application of organics alone and with inorganics significantly improved the mesoaggregate formation compared to the control. Contrarily, the proportion of micro aggregates decreased significantly ($p < 0.05$) with the application of FYM compared with treatments with combination of organics and inorganics (Table 1). This indicated a higher formation of bigger aggregates with the supplementation of organics. Similar results also were observed by Huang *et al.*, (2010) and Bandyopadhyay *et al.*, (2010).

The organic matter is classified as an important binding agent for aggregation and is responsible for the formation and stability of soil aggregates (Tisdall and Oades. 1982) through biotic mechanism (Schjonning *et al.*, 2006). The added organics could supply additional fresh organic residues (water soluble and hydrolysable substrates) and C to the soil resulting in production of microbial polysaccharides that increase aggregate cohesion. This explained the observed progressive increase in aggregate stability to mechanical breakdown. Positive effects of green manure and FYM application on aggregate stability have been reported in a number of studies (Bandyopadhyay *et al.*, 2010; Singh *et al.*, 2007). The proportion of large macro aggregates within the total soil aggregates is the most important fraction to evaluate the effect of management practices on soil aggregation, because it exerts a strong influence on the mean weight diameter (MWD), a comprehensive index for evaluating soil aggregation (Jiao *et al.*, 2006).

Again, higher crop residue-C might have an effect on aggregate stability as plant roots are important binding agents at the scale of macro aggregates (Six *et al.*, 2004). The presence of soil microbial biomass may also influence aggregate formation (Six *et al.*, 2005). FYM applied soils exhibited higher values of aggregate indices. The variations in structural indices among the treated organics might also be influenced by their bio-chemical compositions.

Results showed that the mean weight diameter was significantly ($p < 0.05$) higher in soils under FYM (2.08 mm) compared to other treatments. Geometric mean diameter (GMD) also exhibited similar trend which ranged from 1.32 to 1.42 mm. Aggregate ratio (AR) showed similar trend, with the highest values in soils under FYM treatment (8.76) but lowest values in soil under control (2.40). Aggregate stability (%) was significantly ($p < 0.05$) higher in soils under FYM (93.8%) compared to control, but at par with other treatments. FYM treated soils showed significantly higher MWD, GMD, AR and AS than those under the other treatments (Table 2). Gupta Choudhury (2011) and Datta (2016) also reported similar findings.

Aggregate associated carbon fractions

The aggregate associated C in different sized fractions is presented in Table 4. Incorporation of organics like GM and FYM alone and with inorganics significantly ($p < 0.05$) increased C concentration in different sized aggregates over the other treatments. The maximum amount of SOC was retained in coarse micro (100-250 µm) and fine micro aggregate (53-100 µm) sized fractions followed by > 2000 µm and 500-1000 µm sized fractions. Total macroaggregate associated carbon was higher under RDF+FYM, while total micro aggregate associated carbon was higher under FYM treatments.

Table.1 Influence of treatments on distribution of water stable aggregates into different size fractions at surface layer (0-15 cm)

Treatment	% Water stable aggregates						
	CMacA > 2000 µm	MesoA			Total macro aggregates	CMicA 250-100 µm	Total water stable aggregates
		2000-1000 µm	1000-500 µm	500-250 µm			
Control	67.33 ^c	0.33 ^e	1.23 ^{ab}	1.05 ^{ab}	69.94 ^b	0.51 ^{bc}	70.45 ^c
50% RDF	79.77 ^{ab}	2.18 ^{bcd}	1.03 ^{bc}	0.58 ^{bc}	83.55 ^a	1.01 ^b	84.56 ^b
50% RDF+ GM	86.03 ^a	1.39 ^{cde}	0.87 ^{bc}	0.49 ^{bc}	88.78 ^a	2.02 ^a	90.80 ^{ab}
50%RDF+FYM	82.38 ^a	3.76 ^a	1.86 ^a	0.88 ^{ab}	88.88 ^a	1.08 ^b	89.96 ^{ab}
50%RDF+GM+FYM	84.40 ^a	0.82 ^{de}	0.78 ^{bc}	0.15 ^c	86.15 ^a	1.04 ^b	87.18 ^{ab}
FYM	85.36 ^a	2.23 ^{bc}	0.88 ^{bc}	0.57 ^{bc}	89.04 ^a	0.83 ^{bc}	89.87 ^{ab}
RDF+FYM	88.01 ^a	3.03 ^{ab}	0.35 ^c	1.41 ^a	92.80 ^a	2.06 ^a	94.86 ^a
N	70.80 ^{bc}	0.91 ^{cde}	1.15 ^{ab}	0.93 ^{ab}	73.79 ^b	0.19 ^c	73.98 ^c
RDF	82.56 ^a	1.71 ^{bcde}	0.90 ^{bc}	0.65 ^{bc}	85.82 ^a	0.45 ^{bc}	86.27 ^{ab}

Different small letters within the same column show the significant difference at p = 0.05 according to Duncan Multiple Range Test for separation of mean

Table.2 Influence of treatments on aggregate indices of experimental soils at 0-15 cm soil depth

Treatment	MWD (mm)	GMD (mm)	Aggregate Ratio	Aggregate Stability (%)
Control	1.94 ^c	1.32 ^d	2.40 ^c	54.35 ^b
50% RDF	1.98 ^{ab}	1.35 ^{bc}	5.10 ^{abc}	87.22 ^a
50% RDF+ GM	2.01 ^b	1.37 ^b	9.39 ^a	93.28 ^a
50% RDF+FYM	1.96 ^c	1.34 ^{cd}	8.00 ^a	92.63 ^a
50% RDF+GM+FYM	2.01 ^b	1.36 ^{bc}	7.04 ^{abc}	92.66 ^a
FYM	2.08 ^a	1.42 ^a	8.76 ^a	93.81 ^a
RDF+FYM	1.98 ^{ab}	1.35 ^{bc}	8.14 ^a	68.10 ^b
N	1.96 ^{ab}	1.33 ^{cd}	2.93 ^{bc}	90.93 ^a
RDF	1.99 ^{ab}	1.35 ^{bc}	7.21 ^{ab}	92.05 ^a

Different small letters within the same column show the significant difference at p = 0.05 according to Duncan Multiple Range Test for separation of mean

Table.3 Percent distribution of aggregate associated C into different aggregate fractions and percent change over 50% RDF (values within the bracket)

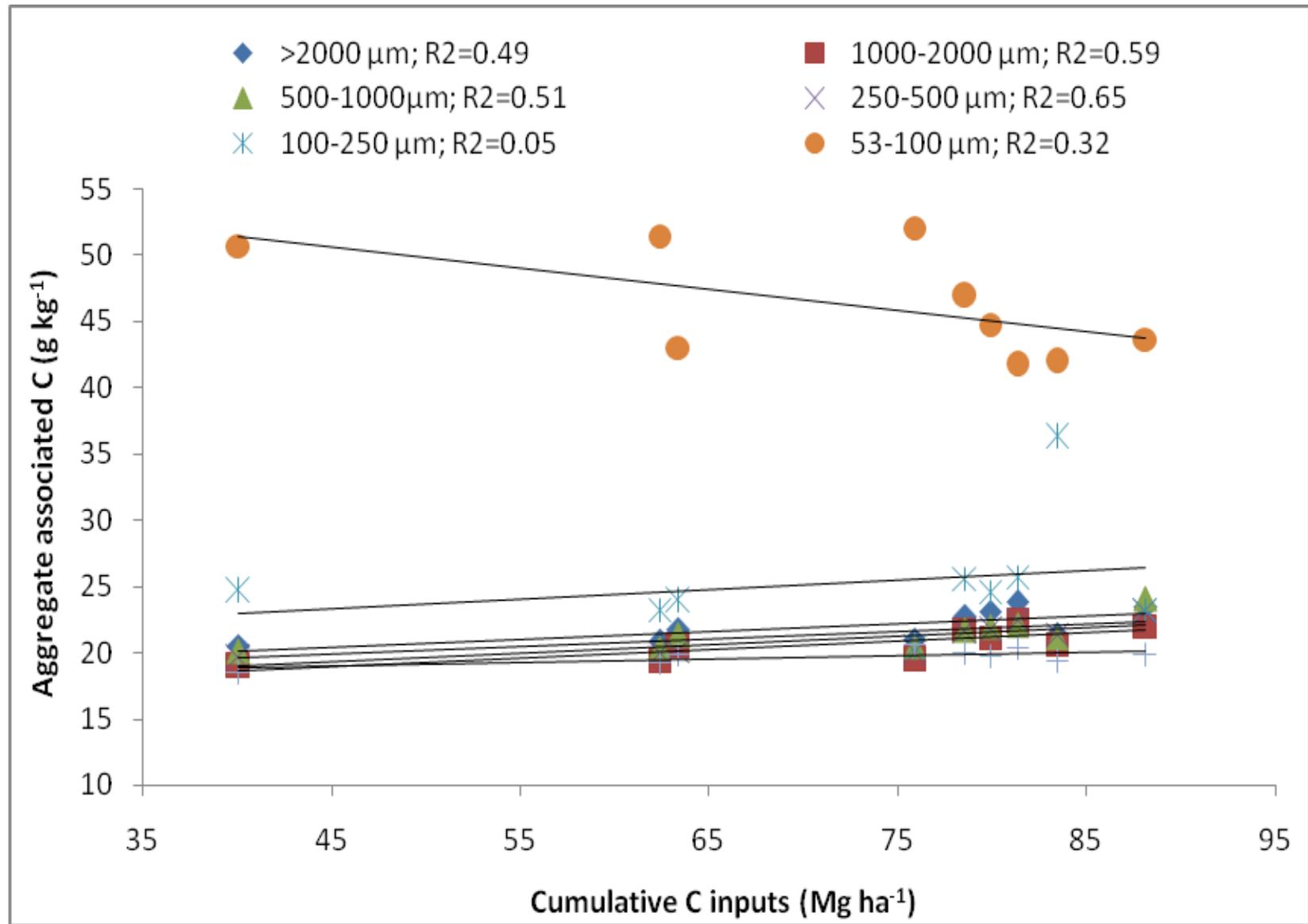
Treatment	% Total aggregate associated C		
	Cmac AC	MesoAC	micAC
50% RDF	12.7	36.3	51.0
50% RDF+ GM	12.6 (4.69)	36.1 (5.10)	51.3 (6.54)
50% RDF+FYM	13.3 (9.69)	37.5 (8.03)	49.2 (4.10)
50% RDF+GM+FYM	13.1 (6.57)	36.6 (4.69)	50.3 (2.65)

Table.4 Aggregate associated C under different size fractions under different treatment

Treatment	Size fraction (µm)								
	Macro aggregated C (g kg ⁻¹)				Micro aggregated C (g kg ⁻¹)				
	CMacAC > 2000	MesoAC			Total Macro AC	CMicAC 100-250	FMicAC 53-100	(Silt+clay) AC < 53	Total Micro AC
		1000-2000	500-1000	250-500					
Control	20.53 ^c	18.97 ^d	20.06 ^c	19.61 ^c	79.17 ^d	24.65 ^b	50.72 ^a	18.56 ^c	93.94 ^a
50% RDF	21.68 ^{bc}	20.46 ^{bcd}	21.30 ^{bc}	20.12 ^c	83.57 ^{bcd}	23.94 ^b	43.04 ^a	19.85 ^{ab}	86.83 ^a
50% RDF+ GM	22.70 ^{ab}	21.78 ^{ab}	21.70 ^b	21.56 ^b	87.75 ^{ab}	25.57 ^b	46.94 ^a	19.99 ^{ab}	92.50 ^a
50%RDF+FYM	23.78 ^a	22.29 ^a	22.09 ^b	22.47 ^{ab}	90.64 ^a	25.70 ^b	41.74 ^a	20.35 ^{ab}	87.79 ^a
50%RDF+GM+FYM	23.11 ^a	21.14 ^{ab}	21.95 ^b	21.70 ^b	87.90 ^{ab}	24.58 ^b	44.76 ^a	19.78 ^{ab}	89.13 ^a
FYM	21.32 ^c	20.67 ^{abc}	21.14 ^{bc}	21.38 ^b	84.51 ^{bc}	36.26 ^a	41.99 ^a	19.31 ^{bc}	97.56 ^a
RDF+FYM	23.51 ^a	22.02 ^{ab}	24.01 ^a	23.08 ^a	92.62 ^a	23.22 ^b	43.66 ^a	19.92 ^{ab}	86.81 ^a
N	20.80 ^c	19.33 ^{cd}	20.41 ^c	20.02 ^c	80.55 ^{cd}	23.24 ^b	51.45 ^a	19.26 ^{bc}	93.95 ^a
RDF	21.01 ^c	19.48 ^{cd}	20.47 ^c	19.99 ^c	80.95 ^{cd}	20.32 ^b	52.08 ^a	20.59 ^a	92.99 ^a

Different small letters within the same column show the significant difference at p = 0.05 according to Duncan Multiple Range Test for separation of mean

Fig.1 Relationship between cumulative carbon inputs and C associated with different sized aggregates



Of the total aggregate associated C, micro aggregates (< 250 µm) had higher amounts (51.7%) compared to macro aggregates (> 250 µm, 48.3%). Application of organics increased C accumulation in different aggregates, the effect was more pronounced with microaggregates than macroaggregates.

Irrespective of the treatments, a maximum amount of C was found with mesoaggregate fractions (250- 2000 µm, 58.64-69.11 g kg⁻¹) followed by fine micro aggregates (53-100 µm, 41.74-52.08 g kg⁻¹), coarse micro aggregates (100-250 µm, 20.32-36.26 g kg⁻¹), coarse macro aggregates (> 2000 µm, 20.53-23.78 g kg⁻¹) and (silt + clay) sized aggregates (< 53 µm, 18.56-20.59 g kg⁻¹) in a decreasing order, constituting, on an average, 35.8, 26.2, 14.3, 12.5 and 11.2%, respectively, of the total aggregate associated C (Table 4).

Aggregate associated C strongly influences C sequestration and dynamics of C cycling in soils. Following 24 years of continuous cropping with different management practices, the experimental soils demonstrated preferential sequestration of SOC in the meso-aggregate fraction(250-2000 µm).

In fact, such sequestration was more with particles of decreasing sizes. A higher surface area for smaller particles may be responsible for this. Christensen (1986) and Kong *et al.*, (2005) also reported similar results. Gupta Choudhury (2011) and Datta (2016) also reported similar findings in Indian subcontinent.

An attempt was made to find out if application of organics in the form of green manure, farmyard manure or their combination could influence the distribution of carbon among macro, meso and micro sized aggregates. On an average, about 51, 36 and 13% of the aggregate associated C was allocated to micro, meso and macro sized

fractions. These values, however, did not change significantly due to the application of any of the above three sources of organic applied. Results thus indicated that applied carbon found its way to different size aggregates proportional to their native distribution in soils (Table 3).

A positive linear relationship was observed between the cumulative C inputs into the soils (during the whole period of experimentation) and the aggregate associated C (Fig 1). Such relationship was stronger particularly with the C associated with the aggregate size fractions of 250-500 µm (R²=0.65). This indicated that smaller particles with greater surface area may be responsible for scavenging a sizable amount of C in micro aggregates. Kong *et al.*, (2005) and Majumder *et al.*, (2007) also reported similar results. However, there was a little influence of increasing C inputs was observed for C associated with aggregates of 250-1000 and < 53 µm size.

Application of organics had immense effect on soil aggregation and C sequestration. Mesoaggregates shared the maximum proportion of aggregate associated C (35.8%) followed by fine micro, coarse micro, coarse macro aggregates and silt + clay fractions constituting 26.2, 14.3, 12.5 and 11.2%, respectively.

Application of organics has no effect on relative distribution of aggregate associated carbon into different size fractions. Thus, it can be concluded that even under hot semi-arid region of the country, growing of double rice system in *Inceptisol* with balanced fertilization or C supplementation through FYM/GM caused a net C sequestration. Hence, in this regions external application of carbon through different organics (FYM/GM) is necessary to improve carbon content in soils and maintain sustainable crop production/yields.

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