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Distribution of soil organic carbon within water stable soil Aggregates in long-term fertilized and Manured Rice-Wheat rotation

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Abstract
An experiment was conducted in a 22nd cycle of long-term rice-wheat rotation established in the hot humid subtropics of eastern India to evaluate carbon concentration within water stable aggregates in soils undergoes influences of continuous manuring and fertilization. Soil samples were collected at 0-15cm depth from twelve treatments with different type of organic amendments such as farmyard manure, paddy straw and green manure with two levels of Nitrogen substitution (50% and 25%) from. The wet sieving technique was employed to separate soils and catagorized into >2.0, 2.0-0.25, 0.05-0.25 and<0.05mm aggregate fractions. There was an enrichment of organic carbon content in the larger size aggregates (>2 mm) compared to smaller aggregates (<0.25 mm). Higher proportion of organic carbon was occluded within small macro aggregates (0.25-2.0mm) than fine micro aggregates (0.05-0.2mm) as well as silt plus clay sized soil separates (<0.05mm). The effects were more pronounced in organically amended soils rather than unfertilized and solely fertilized soil. Within a size class, aggregated C concentrations of the organically amended treatments were in the order of FYM>PS≥GLM. In all the cases 50% substitution of N by organic amendments recorded much higher values than 25% substitution of N.

Keywords: aggregates, organic amendments, rice-wheat, soil organic carbon

Introduction
The rice-wheat cropping considered as a one of the largest agricultural production system in the world which contributing nearly one-third of the cultivable area under both the crop grown in South Asia (12Mha, in India) (Kumari *et al*., 2011) [9]. But in the recent past such production system undergoes major yield loss due to gradual depletion of organic matter, soil nutrients and decline in soil physical productivity (Mitran *et al*.,2016a; Gupta *et al*., 2003;Manna, 2005) [18]. Rice-wheat system mostly characterize by wetland culture i.e. puddling which results in poor structure and physical condition of the soil for the successive crop (Sharma *et al*., 2003; Bandyapadhyay *et al*., 2010).Although there is a provison to restore the physical condition of such soils by means of aggregation. The enhancement of organic matter content in such soil may be the possible and feasiable option to improve soil aggregation which can be achieve by long-term application of manures. There is much evidence in the literature that the deterioration of soil physical structure can be offset with the long-term application of various organic amendments such as manure, compost, and crop residues etc (Sharma & Bhushan, 2001; Mandal., 2007). The long-term repeated application of organic manure can enhances soil organic carbon level (Maillard & Angers, 2014) which has a positive impact on soil aggregation too (Yu *et al*., 2012). Aggregation is a means to both conserve and protect soil organic matter and allow it to function as a reservoir of plant nutrient and energy. Aggregation has major effects on carbon cycling in soil, contributes to fertility and reduces soil erosion.

On the other hand a proper understanding of carbon dynamics requires an evaluation of the locations of carbon within aggregates too (Bandyapadhyay *et al*., 2010). The characterization of water stable aggregates and allocation of organic carbon within its is very important to understand carbon cycling in soil and mechanism of soil erosion also.The distribution of soil organic carbon in different aggregate size classes may affect erosion, and more rapid loss may occur from macro aggregates than micro aggregates (Eynard *et al*., 2006). As different aggregate fractions are selectively removed during erosion, characterization of these aggregates is needed in understanding carbon dynamics during fertility erosion. Several researcher across the world have studied the long-term effects of manuring and fertilization on distribution of water stable aggregates and associated caron in it (Wang, 2014; Rasool *et al*., 2008; Lugato *et al*., 2010; Bandyapadhyay *et al*., 2010). Long-term manuring has an impact on regeneration and rejuvenation of soil structure. Therefore knowledge of soil aggregate distribution is very much required in the evaluation of soil physical environment as a function of organic amendments. It is hypothesized that the long-term rice-wheat cropping rotation with regular incorporation of various organics i.e. manure, vermicompost, green leaf manuring etc. may have an impact on the water stable aggregates as well as the distribution of organic carbon in different aggregate size classes and hence a long-term fertility experiment of rice-wheat system has been selected for the current study. A better understanding of the effect of long-term manuring and fertilization on organic carbon distribution within water stable aggregates in rice-wheat cropping system will enable a more realistic evaluation of erosion potential, poor yield and sustainability of rice-wheat cropping system.

Materials and methods

Details of the experimental site

Soil samples were collected from a ongoing long- term field experiment (Since 1986) with a rice (*O. sativa L.*, *cv* IET 4094) - wheat (*T. aestivum L.*, *cv* UP 262) cropping system. The field is located (22°57'29.16"N and 88°29'5.91"E) at the University Teaching Farm, Bidhan Chandra Krishi Viswavidyalaya, India.According to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014) the experimental soil is classified as Anthropic HyperarenicCambisols (Stagnic, Dystric) (Mitran *et al*., 2016b) [18]. The major characteristics of the long-term experiment are presented in Table 1.

Treatments Details

The details of the treatment is presented in Table.2.

Soil sampling and analysis

In the present investigation we have followedsame soil sampling protocol as used by Majumder *et al*. (2008) [14] and same fractionation protocol of soil aggregates used by Bandyapadhyay *et al*. (2010) and Mitran *et al*. (2017b) [16, 17] for the same experimental field. Although they have collected soil sample from treatment having 50% substitution of Nitrogen by Farm Yard manure (FYM), Green manure (GM) and paddy straw (PS) along with control, conventional practicesand inorganically treated plots. In the current study we have considered 25% substitution of Nitrogen by FYM, GM and paddy straw also.

Soil aggregate analysis and aggregate associated organic carbon

Aggregate analysis was carried out using Yoder wet sieving apparatus (Kemper and Rosenau, 1986) and then aggregates were fractioned into water stable macroaggregates (WSMacA, >0.25 mm) and microaggregates (WSMicA, <0.25 mm) categories. Further, sieves ranged from 2.0-0.05 mm size were used for separation of four soils aggregate size classes, large Macroaggregates (>2.0 mm), small macroaggregates (0.25-2.0 mm), fine microaggregates (0.05-0.25 mm) and 'silt + clay' sized fraction (<0.05 mm). The organic Cconcentrationin different aggregate size fractions was measured by wet oxidation using a hot mixture (at 170ºC for 30 min) of 1N potassium dichromate and concentrated sulphuric acid following the method of Yeomans and Bremner (1989). The cumulative concentration of organic C exsist in soil aggregate size fractions of >2.0 mm, 0.25–2.0 mm, 0.05–0.25 mm and <0.05 mm were categorized as large macroaggregatedC (LMacAC), small macroaggregatedC(SMacAC), Fine microaggregatedC (FMicAC) and 'silt + clay' associated C (SCAC), respectively(Bandyapadhyay *et al*., 2010). The sum of C in >0.25 mm aggregate size fractions was considered asmacroaggregated C (MacAC), while that in <0.25 mm size fractions was catagorized as microaggregated C (MicAC).

Table 1: Detailed background information of the experiment

Item	Characteristics
Geographical location	Latitude - $22^0 58' 20''$N, Longitude- $88^0 30' 11''$E, Altitude - 9.75 m (msl)
Year of start	*Kharif*, 1986
Climate	Sub-humid
Rainfall	1576 mm
Mean annual temperature (^{0}C)	Max. 29.2 and Min. 18.5
Relative humidity (%)	Max. 98.0 and Min 65.5
Sunshine hours	6.95
No. of rainy days	146
Soil	*AericHaplaquept*, Texture –clayey
Agro-ecological zone	15.1
Agro-ecosystem	Irrigated
Design of experiment	Randomized Block Design, with four replications
Plot size	8.0 m X 8.0 m
Cropping system	Rice-wheat
Crop variety used	Rice – IET 4094 (IET 1444 up to 1997) and, wheat – UP-262 (Sonalika, up to 1994)
Spacing	Rice – 20 cm X 10 cm, wheat – 20 cm solid row
Recommended fertilizer dose	Rice - N : P : K :: 80 : 40: 40, and, wheat - N : P : K :: 100 : 60: 40

(***Source***: Mitran *et al*. 2017a) [16, 17]

Statistical analysis

The statistical significance test has been carried out to evaluate the performance of the treatments using Windows-based SPSS program (Version 10.0, SPSS, 1996, Chicago, IL). The 5% probability level has been considered as statistically significant.

Table 2: Treatment details

Treat	*Kharif*	*Rabi*
T_1	No fertilizers, no organic manure (control)	No fertilizers no organic manure (control)
T_2	50% recommended NPK dose through fertilizers	50% recommended NPK dose through fertilizer.
T_3	50% recommended dose NPK through fertilizers.	100% recommended NPK dose through fertilizers
T_4	75% recommended NPK dose through fertilizers	75% recommended NPK dose through fertilizers
T_5	100% recommended NPK dose through fertilizers	100% recommended NPK dose through fertilizers
T_6	50% recommended NPK dose through fertilizers+ 50% N through Farm Yard Manure	100% recommended NPK dose through fertilizers
T_7	75% recommended NPK dose through fertilizers 25% N through Farm Yard Manure	75% recommended through NPK dose through fertilizers
T_8	50% recommended NPK dose through fertilizers + 50% N through Paddy Straw	100% recommended NPK dose through fertilizers
T_9	75% recommended NPK dose through fertilizers + 25% N through Paddy Straw	75% recommended NPK dose through fertilizers.
T_{10}	50% recommended NPK dose through fertilizers + 50%N through green organic matter (Green Manuring)	100% recommended NPK dose through fertilizers
T_{11}	75% recommended NPK dose through fertilizers + 25% N through green organic matter (Green Manuring)	75% recommended NPK dose through fertilizers
T_{12}	Conventional farmers practice (the amount of fertilizers used in New alluvial zone (50:30:20)	Farmers' practice (conventional) (60:20:20)

(On an average the nutrient content on dry weight basis is as follows (Moisture level 20.0%) of FYM (N: P: K=0.58%, 0.36%, 0.3%), PS (N, P, K=0.81: 0.22: 0.52), GM (N: P: K=2.65%, 0.16%, 0.46%) during the experimental period (Mitran *et al*., 2016) [16].

Results

Proportion of water stable aggregates among the treatment

Results revealed that soilstreated with organic amendments (NPK + FYM, NPK + PS and NPK + GM) showed more water stable macroaggregates (WSMacA). The Cultivation with organics produces more macro aggregates and total water stable aggregates with the following efficiency FYM>PS> GLM. The treatment with 50% substituation of organic materials (NPK+FYM, NPK+PS and NPK+GM) showed a higher proportion (64.4-72.9%) of the water stable macro aggregates (>0.25 mm) and a lower proportion (27.1-35.6%) of the micro water stable aggregates (<0.25 mm) than those without supplemented by organic amendment(Control and NPKs).However 25% N supplement through organicsalso showed similar trend but the effect was comperatively less compared to 50% of nitrogen substitutions.

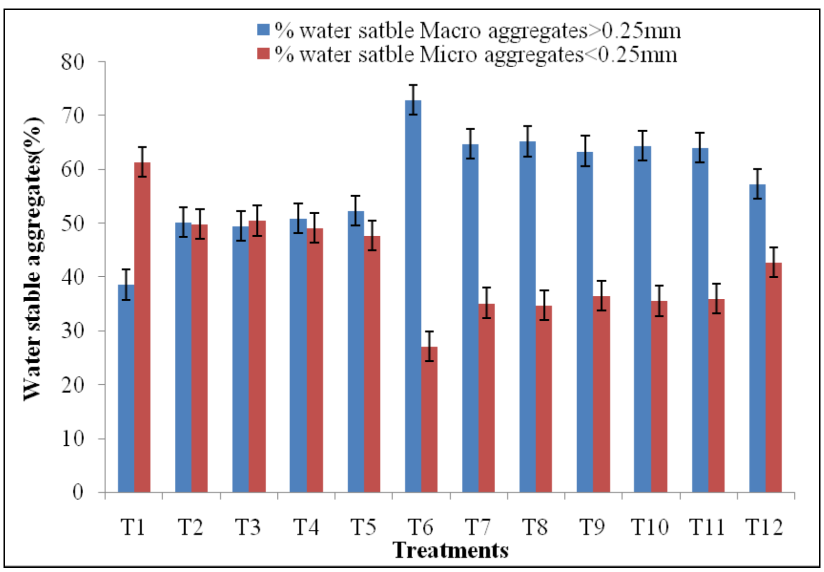

Fig 1: Influence of Organic amendments on distribution of Water Stable Macro and Micro Aggregates (Note:T_1–control (no fertilizer no organic manure); T_2–50% recommended dose of fertilizers (RDF) to both rice and wheat; T_3– 50% RDF to rice and 100% RDF to wheat; T_4– 75% RDF to both rice and wheat; T_5– 100% RDF to both rice and wheat; T_6– 50% RDF + 50% N through farm yard manure (FYM) to rice and 100% RDF to wheat; T_7– 75% RDF + 25% N through FYM to rice and 75% RDF to wheat; T_8– 50% RDF + 50% N through paddy straw to rice and 100% RDF to wheat; T_9– 75% RDF + 25% N through paddy straw to rice and 75% RDF to wheat; T_{10}– 50% RDF + 50% N through green manuring to rice and 100% RDF to wheat; T_{11}– 75% RDF + 25% N through green manuring to rice and 75% RDF to wheat; T_{12}– Conventional farmer's practice).

The treatment with 25% replacement of N via (NPK+FYM, NPK+PS and NPK+GM) showed (63.4-64.8%) water satblemacro aggregates and (35.2-36.6%) water stable micro aggregates (Figure 1).

Distribution of organic carbon within water stable aggregates

The distribution of organic carbon within the water stable aggregates (>2 mm to 0.05 mm) in the experimental soil under different treatments were varied significantly. Results showed that, there was a preferential enrichment of organic carbon content in the larger size aggregates (>2 mm) compared to the smaller size aggregates (<0.25 mm). The trend of distribution of organic carbon within the water stable aggregates indicated decreases in organic carbon content with decreases in aggregate fractions. Among the treatment combination, treatments having organic residues (FYM, paddy straw and greenmanures) in combination with NPK fertilizers showed higher concentration of organic carbon within the >2 mm aggregates compared to control and 100% NPK fertilizers (Figure 2). But the effect was more pronounced with 50% subsituition through FYM, paddy straw and green manure as compared to 25% level of replacement of Nutrogen using such organic amendments.

Impact of organic amendments on macro aggregated carbon

Coarse macro (>2 mm) and small macro (0.25- 2 mm) aggregated carbon collectively contributes towards the macro aggregated carbon pool. Coarsemacroaggregated carbon (CMAC) contributed 26.3% of total macro aggregated carbon. The CMAC values ranged from 4.8 g kg^{-1} to 6.45 g kg^{-1} under different treatments contributing 25.6% and 25.2% respectively of the total CMCA (Table 3). On average the treatments having only NPK fertilizers at different rate were at par. But the higher organic carbon content observed in the treatments having organic residues in combination with inorganic fertilizers. The highest carbon values were associated with the 50% NPK plus 50% N through FYM (6.45 g kg^{-1}) followed by 50% NPK plus 50% N through PS (6.15 g kg^{-1}) and 50% NPK plus 50% N through GM (5.40 g kg^{-1}). Among the treatments T_6 (50% NPK plus 50% N through FYM) contributed 34.3% & 22.8% more organic carbon over control and 100% NPK respectively in the coarse macro aggregates. From the results it was observed that higher organic carbon accumulated within the small macro aggregates (0.25 to 2 mm) than other aggregates and it contributed higher proportion (73.7%) of total macro aggregated carbon. The highest and lowest values of organic carbon within small macro aggregates were associated with the treatment T6 (19 g kg^{-1}) and T1 (13.9 g kg^{-1}) respectively. Here again also the aggregated fractions under organic amended soils showed their relative dominancy over 100% NPK and control. The proportion of organic carbon in the small macro aggregated fractions increased with the application of 50% NPK plus 50% N through FYM, paddy straw, green manures over control and 100% NPK fertilizers. The magnitude of increases was higher in FYM treated soil (49.6%) followed by paddy straw (16.5%) and green manures (13%) respectively over control.

Impact of organic amendments on micro aggregated carbon

Fine micro aggregated (0.05-0.25 mm) and silt plus clay associated organic carbon collectively contributes towards the micro aggregated organic carbon. From graphical presentation (Figure 2) it is clear that organic carbon sequestered from 2.0 mm above to 0.25-0.1 mm size of the aggregates. After that organic carbon could not be sequestered by very fine aggregates. All the organic amendments have similar effects C distribution in aggregate fraction. In the present experiment it was found that on average fine micro aggregated organic carbon contributed 58.7% of total micro aggregated organic carbon in the experimental soils under different treatments. From the Table 3 it has been found that highest organic carbon content in the fine micro aggregates associated with the T_6 (50% NPK plus 50% N through FYM), where as lowest value observed in the control treatment. Results showed that the organic carbon content in the fine micro aggregates increased by 72% in T_6 (50% NPK plus 50% N through FYM), 34.8% in both T_8 (50% NPK plus 50% N through PS) and T_{10} (50% NPK plus 50% N through GM) respectively over control.

Impact of organic amendments on silt plus clay sized organic carbon

The results of present investigation indicated that silt plus clay sized organic carbon contributed 41.2% of total micro aggregated organic carbon. Here also the application of organic residues combined with fertilizers significantly increases the silt plus clay associated organic carbon over control and only fertilizers application. From the results it has been found that T_8 (50% NPK + 50% N through PS) contains higher proportion of organic carbon (29%) within silt plus clay sized fractions over control but there was no significant difference existed between T_6, T_8 and T_{10} (Table 3).

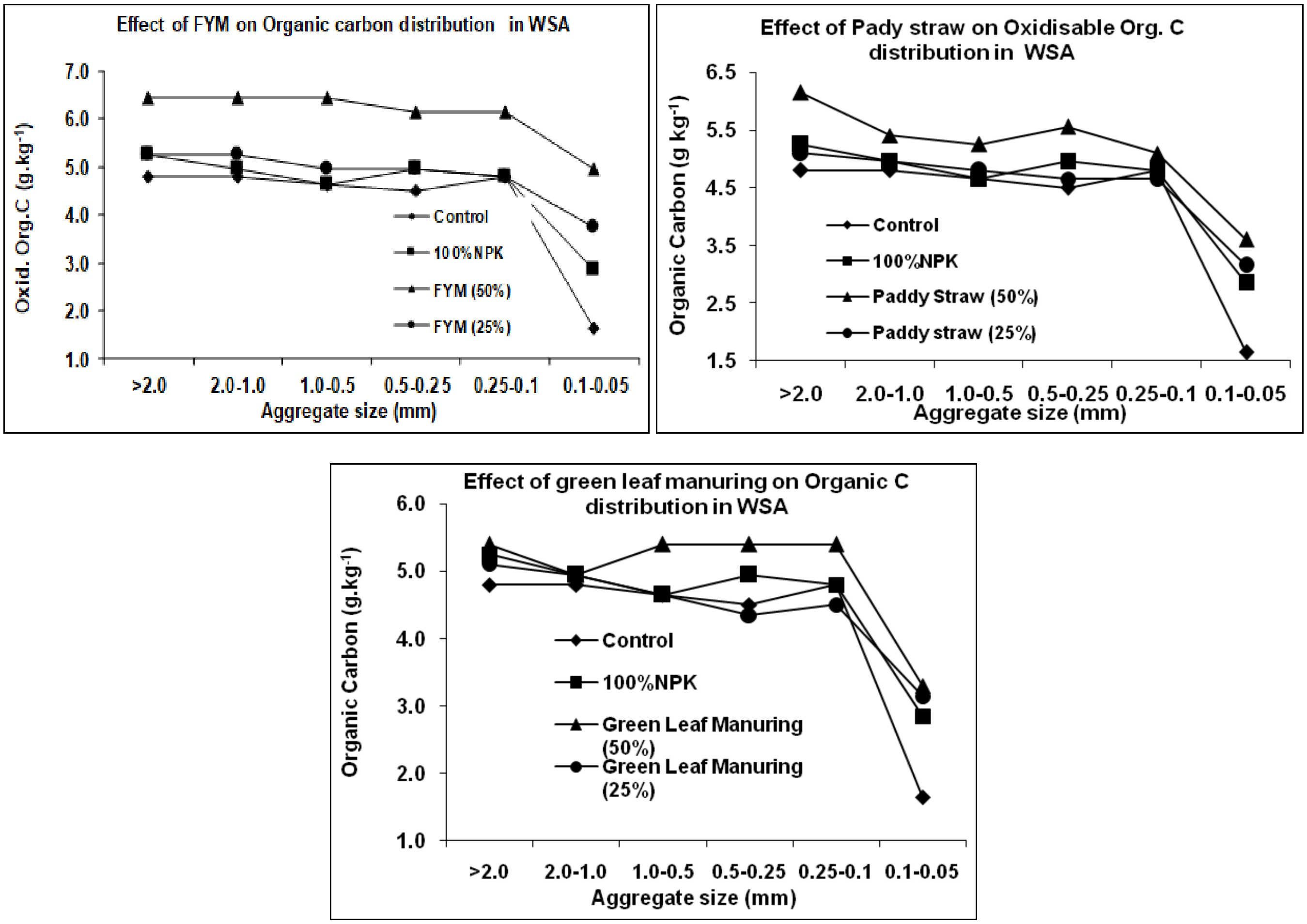

Fig 2: Distribution of Organic Carbon within water stable aggregatesunder various treatments

Table 3: Long term manuring and fertilization influences on allocation different aggregated C (g kg^{-1}) under different treatments

Treatment	**(>2.0 mm)**	**(0.25-2.0 mm)**	**(0.05-0.25 mm)**	**(<0.05m)**	**Total (>0.25 mm) macro-aggregated**	**Total (<0.25 mm) micro-aggregated**
	Large macro-aggregated	**Small macro-aggregated**	**Fine micro-aggregated**	**Silt + clay Sized**		
T_1	4.80	13.9	6.45	5.00	18.7	11.4
T_2	5.25	13.3	6.15	4.90	18.6	11.0
T_3	5.25	13.5	6.00	4.85	18.7	10.8
T_4	4.95	14.7	6.90	4.20	19.6	11.1
T_5	5.25	14.5	7.65	5.30	19.8	12.9
T_6	6.45	19.0	11.1	6.20	25.5	17.3
T_7	5.25	15.1	8.55	6.30	20.4	14.8
T_8	6.15	16.2	8.70	6.45	22.3	15.1
T_9	5.10	14.4	7.80	6.45	19.5	14.2
T_{10}	5.40	15.7	8.70	6.25	21.1	14.9
T_{11}	5.10	13.9	7.65	5.20	19.0	12.8
T_{12}	4.95	14.2	6.90	3.80	19.2	10.7
Mean	5.33	14.9	7.71	5.40	20.2	13.1
S.Em(+)	0.24	0.62	0.22	0.35	0.36	0.28
LSD (P=0.05*)	0.69	1.82	0.64	1.04	1.03	0.84

(Note:***significant at 5% level**,T_1–control (no fertilizer no organic manure); T_2–50% recommended dose of fertilizers (RDF) to both rice and wheat; T_3– 50% RDF to rice and 100% RDF to wheat; T_4– 75% RDF to both rice and wheat; T_5– 100% RDF to both rice and wheat; T_6– 50% RDF + 50% N through farm yard manure (FYM) to rice and 100% RDF to wheat; T_7– 75% RDF + 25% N through FYM to rice and 75% RDF to wheat; T_8– 50% RDF + 50% N through paddy straw to rice and 100% RDF to wheat; T_9– 75% RDF + 25% N through paddy straw to rice and 75% RDF to wheat; T_{10}– 50% RDF + 50% N through green manuring to rice and 100% RDF to wheat; T_{11}– 75% RDF + 25% N through green manuring to rice and 75% RDF to wheat; T_{12}– Conventional farmer's practice).

Discussion

Result of the study revealed that there is a decreases in organic carbon content with decreases in aggregate fractionsi. emacroaggregatses showed higher concentration of organic carbon compared to micro aggregates. The effects were more pronounced with organically treated plot. Other studies have similarly reported greater macro-aggregated C proportionally to manure addition (Annabi *et al*., 2011; Liu *et al*., 2014; Spaccini *et al*., 2001; Sui *et al*., 2012; Fang *et al*., 2014) [1, 10, 23, 24]. According to Jastrow *et al*. (1996) the formation of aggregate associated carbon in small aggregates occurs more quickly than in large aggregates, but ultimately SOC content in large aggregates is greater than in small aggregates. The micro aggregates formed within macro-aggregates could contribute significantly to SOC stabilization (Six *et al*., 2000) [22]. The view of the current study is also supported by the fact reported by Cambardella & Elliot, 1993; Tisdall & Oades, 1982 [25]. They found that the polysaccharides, polyuronides and phenols were more associated with the >0.25 mm water stable aggregates. Fine micro aggregated organic carbon existed in between 0.05 to 0.25 mm sieve size fraction and are resistant to microbial degradation (Six *et al*., 2000) [22]. Carbon accumulation in the fine micro aggregates fractions not only as a result of C loading through organics but also due to a transfer of carbon from macro aggregated carbon. The bio polymers (e.g. lignin, polyphenols etc.) derived from roots and hyapae as well as added organics exhibit a high degree of resistance to microbial degradation as compared to cellulose poly- saccharides in macro aggregated carbon and seemed to be stabilized in fine micro aggregates (Bandyopadhyay *et al*., 2010). The silt plus clay associated carbon was formed during decomposition of macro aggregated carbon and stabilization through soil organic carbon binding with clay minerals and is a very important pool for the total soil organic carbon. The silt plus clay sized organic carbon held on the mineral surfaces by several interaction mechanism such as ligand exchange, H-bonding, hydrophobic bondinds and others. On the other hand the variation in organic carbon content in the coarse macro aggregates, under different organic residues added, due to the fact that the total carbon content of the individuals organics and their degree of decomposition. Low content of cellulose in green manures (10%., Majumder *et al.*, 2008) [14] may be the cause for least small macro aggregated carbon in the treatment containing green manures. So, the overall results therefore indicated that the organic carbon content in the micro aggregates (<0.25 mm) was less than that of water stable macro aggregates (>0.25 mm) under the influence of long-term application of manures & fertilizers in the experimental soil. Differences in macro aggregated carbon and micro aggregated carbon would be governed by interplay of several factors including climate, soil characteristics, substrate, biochemistry, C loading and associated environments. From this study it can be concluded that there was a greater accumulation of aggregate associated organic carbon due to the supplementation of FYM, paddy straw and green manuring with NPK that added organic carbon and increased root biomass.

Conclusion

The distribution pattern of C showing preferential enrichment of the larger aggregates than the smaller aggregates. However micro aggregated C has more physically protected and therefore more biochemically recalcitrant. Cultivation leads to micro aggregates which is more susceptible to erosion that could be checked by application of organics like FYM, PS, GM that increased the mass of water stable aggregates through accumulation of micro aggregates.

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Abbreviation

C: Carbon, FYM; Farm Yard Manure, FMicAc; Fine Micro aggregated Carbon, FYM; Farm Yard Manure, GM; Green Manuring, GMD; Geometric Mean Diameter, MWD; LMacAC; Large Macro aggregated Carbon Mac AC; Macro aggregated Carbon, MicAC; Micro aggregated Carbon, PS; paddy straw, SMacAC; Small Macro aggregated Carbon, SCAC; Silt + Clay associated Carbon, WSA; Water Stable Aggregates, WSMacA; Water Stable Macro aggregates, WSMic A Water Stable Micro aggregates.

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