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Effect of long term manuring and fertilization on soil aggregation under rice-wheat cropping system in an Inceptisol

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Abstract
The long-term effect of manuring and fertilization on aggregate distribution and aggregate associated C was studied under the rice-wheat cropping system in an Inceptisol of the upper Indo-Gangetic plain. Eight treatments from the long term experiment were chosen for this study: control (T1), 100% NPK-fertilizer (T2), 50% NPK- fertilizer + 50 % N-FYM (T3), 75% NPK + 25% N-FYM (T4) and 50% NPK + 50% N-Wheat straw (WS) (T5), 75% NPK + 25% N-WS (T6), 50% NPK + 50% N-Green manure (GM) (T7) and 75% NPK + 25% N-GM (T8). The results revealed that application of balanced chemical fertilizer alone or in conjunction with FYM, WS or GM favoured the soil aggregation and C buildup in the soil. Highest % water stable macro-aggregates (WSMA) (50.49%) and mean weight diameter (MWD) (0.85 mm) were resulted under T3 (50% NPK + 50% N-FYM). Macro-aggregate associated C (MA-C) and micro-aggregate associated C (mA-C) were increased by 48.7 and 126% respectively over control under T6 (75 % NPK + 25 % N- WS). Glomalin content was positively related to the stability of aggregates as indicated by the strong correlation of MWD with glomalin associated with macro-aggregates (GMA) (r=0.74*) and glomalin associated with micro-aggregates (GmA) (r=0.95**).

Keywords: Aggregation, mean weight diameter, rice-wheat cropping, aggregate associated carbon

Introduction
The soil aggregation is an important process of soil organic C (SOC) stabilization by providing different degrees of protection from decomposition by microorganisms (Jastrow *et al*., 1996) [8]. Aggregates physically protect the SOC by forming physical barrier between microorganism plus microbial enzymes and their substrates (Six *et al*., 1999) [15]. Aggregates are formed by flocculation, cementation, and the rearrangement of particles (Duiker *et al*., 2003) [4]. Soil organic matter is the major binding agent in the soils which helps in the formation of aggregates (Tisdall and Oades, 1982) [17]. Organic materials such as humic matter are involved in the stabilization of micro-aggregates and transient bonding agents (polysaccharides) as well as temporary binding agents (fungal hyphae, fine roots, bacterial cells) related to the formation and stabilization of macro-aggregates. Aggregates are categorized into different size classes based on diameter. Commonly three aggregate size classes are defined as large macro-aggregates (>2mm), small macro-aggregates (0.25-2 mm), and micro-aggregates (0.053-0.25 mm), respectively. Due to the small structure and micro-sized pores, micro-aggregates tend to be more stable than macro-aggregates and are key factors in storing SOC for the long-term (Six *et al*., 1998) [14].
Soil aggregation and structure are affected by cultivation under different management practices with or without application of organic and inorganic sources of nutrients for a long period (Sharma and Bhushan, 2001) [13]. Formation of water stable aggregates hence C sequestration is influenced by the types of applied organic manures and crop residues (Mandal *et al*., 2007) [11]. It is important to evaluate the soil aggregation and its stability with regard to the organic amendments as it is useful for evaluation of soil properties and C sequestration. Therefore, in the present study, an attempt was made to examine the long term effect of manuring and fertilization on soil aggregate size distribution and C associated with macro and micro aggregates.

Materials and Methods

Experimental site

The long term fertilizer experiment was started in 1984 at Crop Research Farm, Chandra Sekhar Azad University of Agriculture and Technology, Kanpur (26º58´N, 80º34´E, 129 m above msl) representing Central Plain Zone of Uttar Pradesh. The climate of the experimental site is sub-tropical monsoonal with an annual rainfall of about 818 mm and mean monthly maximum and minimum air temperature of 31.9 ºC and 19.6 ºC respectively, monthly mean relative humidity ranges from 53.5% to 90.3%. Most of the rains received during *Kharif* season from July to September. The surface soil (0-15 cm) of the experimental site was sandy loam in texture with mean particle distribution of 47% sand, 35% silt and 18% clay and was slightly alkaline in reaction (pH= 8.1) and non-saline (EC= 0.18 dS m^{-1}). The initial soil had organic C 2.4 g kg^{-1}, available N 83.0 kg ha^{-1}, available P_2O_5 6.3 kg ha^{-1}, and available K_2O 82.0 kg ha^{-1}.

Soil sampling and processing

Surface soil samples (0-15 cm) were collected from the treatments comprising of control (T_1), 100% NPK- fertilizer (T_2), 50% NPK- fertilizer + 50% N-farmyard manure (FYM) (T_3), 75% NPK + 25% N-FYM (T_4) and 50% NPK + 50% N-Wheat straw (WS) (T_5), 75% NPK + 25% N-WS (T_6), 50% NPK + 50% N-Green manure (GM) (T_7) and 75% NPK + 25% N-GM (T_8). These treatments were imposed only in rice but in wheat all the nutrients were applied through fertilizers at the rate of 120 kg N, 60 kg P_2O_5 and 60 kg K_2O/ ha. The recommended dose of NPK for rice for T_2 (100% NPK) was 120 Kg N, 60 Kg P_2O_5 and 60 kg K_2O/ ha. The treatments were replicated four times and laid out in randomized complete block design having plot size 8m x 8m. At the time of final land preparation green manure (GM) (*Sesbanea aculeata* L.), FYM, wheat straw (WS) and entire phosphatic and potassic fertilizers along with 50% of N were applied as basal dose as per treatment. The remaining 50% N was applied with two equal splits one at tillering and another at panicle initiation stage. One portion of the collected soil samples was air-dried, ground, and sieved to pass through a 4.75-mm sieve and was used for aggregate separation.

Soil analysis

Aggregate stability of the samples was determined by wet sieving methods following Cambardella and Elliot (1993) [2] taking the sieves of 2, 0.25 and 0.053 mm sizes. The different aggregate size fractions obtained were large macro-aggregate (> 2.0 mm), small macro-aggregate (2.0-0.25 mm), micro-aggregate (0.25-0.053 mm) and clay + silt (< 0.053 mm). All the aggregate fractions were oven dried at 60 °C until constant weight. Mean weight diameter (MWD) was calculated from the following equation (Kemper and Koch, 1966) [9]:

$$MWD = \sum X_i * W_i \qquad (1)$$

where Xi was the mean diameter (mm) of the soil aggregate size fractions and Wi was the proportion of each aggregate size with respect to the total sample weight. The percentage weight of water stable macro-aggregates (WSMA) was determined by the sum of aggregates retained on sieves >0.25 mm diameter.

Total soil C (TSC) contents in aggregates were determined by dry combustion method (Nelson and Sommers 1983) [12]. Soil inorganic C (SIC) content was measured by treating the soil with 1N HCl followed by trapping of CO_2 by 0.5 N NaOH (Snyder and Trofymow 1984) [16]. Soil organic C (SOC) was calculated by the difference of TSC and SIC.

The glomalin content of aggregate samples were extracted using 50 mM sodium tricitrate, pH 8.0, 121°C for 1 hr (Wright and Upadhyay, 1998) [18]. Extraction was repeated until the extractant was straw colored. A modified Bradford protein assay (Wright and Upadhyay, 1996) [19] was used to measure glomalin content in the extract. Samples were diluted with PBS (Phosphate Buffered Saline) and reacted with bio- rad, Bradford protein dye reagent. The intensity of colour was read after 5 min at 595 nm in a spectrophotometer. Glomalin content was determined by comparison with a bovine serum albumin (BSA) standard curve.

Statistical analysis

Experimental data obtained were subjected to analysis of variance (one way ANOVA) in completely randomized block design (RBD) using window based statistical software, SPSS version 16.0. The Duncan's Multiple Range Test at probability 5% was used to segregate significance of difference among the mean values. The correlation between WSMA, MWD and glomalin associated with aggregates was also done by the same statistical package.

Results and Discussion

Distribution of soil aggregates: Aggregates distribution, MWD and % WSMA were significantly affected by long-term application of manures and fertilizers in 0-15 cm soil depth (Table 1). The MWD, % WSMA were significantly higher in T_3 (50% NPK + 50% N- FYM) compared to other treatments. The MWD was 77% higher in the T_3 (50% NPK + 50% N- FYM) than control. Increasing the substitution of N from 25 to 50% by organic sources (FYM, WS, GM) resulted in increased aggregate stability as indicated by higher values of MWD and WSMA. All the integrated treatments resulted in a significantly higher value of MWD compared to T2 (100% NPK) as well as control. Among the organic sources FYM was superior over WS and GM. The increase in the MWD may be due to the addition of more organic matter under NPK and integrated treatments. Aggregates are stabilized by the forming and strengthening of the bonds between organic matter and clay domains and between quartz particles and clay domains (Emerson, 1977) [5]. The addition of organic matter to soil might result in the production of microbial polysaccharides which increases the aggregate stability to mechanical breakdown (Bandyopadhyay *et al*., 2010) [1]. The proportion of micro-aggregates was highest under control and it was decreased with the addition of organic manures and residues because added organic matter helps in the formation of macro-aggregates by binding micro-aggregates in the soil (Tisdall and Oades, 1982) [17]. Application of balanced fertilizers from inorganic and organic sources improved the water stable aggregates were also observed in similar field trials (Hati *et al*., 2007; Li *et al*., 2010; Bandyopadhyay *et al*., 2010; Das *et al*., 2014) [7, 10, 1, 3].

Table 1: Long term effect of manuring and fertilization on aggregate size distribution (%), mean weight diameter (MWD; mm) and water stable macro aggregates (WSMA; %) in 0-15 cm soil depth under rice wheat cropping system in Inceptisol

Treatments	Aggregates (%)				MWD (mm)	WSMA (%)
	>2.0 mm	2.0-0.250 mm	0.250- 0.053 mm	< 0.053 mm		
Control (T_1)	4.62^f	20.51^h	71.85^a	3.01^a	0.48^g	25.14^h
100% NPK (T_2)	6.79^e	23.26^g	66.86^b	3.08^a	0.57^f	30.05^g
50% NPK + 50% N- FYM (T_3)	11.06^a	39.44^a	48.49^g	1.02^d	0.85^a	50.49^a
75% NPK + 25% N- FYM (T_4)	7.96^c	30.14^c	60.53^e	1.37^c	0.67^c	38.10^c
50% NPK + 50% N- WS (T_5)	10.43^b	37.23^b	50.89^f	1.44^c	0.81^b	47.66^b
75% NPK + 25% N- WS (T_6)	7.31^d	28.45^d	62.34^d	1.90^b	0.63^d	35.76^d
50% NPK + 50% N- GM (T_7)	8.25^c	26.36^e	63.59^c	1.80^b	0.64^d	34.61^e
75% NPK + 25% N- GM (T_8)	7.37^d	24.36^f	66.27^b	2.00^b	0.60^e	31.73^f

The data followed by different lower case letters in a particular column are significant according to Duncan's Multiple Range Test at P=0.05

Soil organic C associated with aggregates

Macro-aggregate associated carbon (MA-C) varied significantly across various treatments in the 0-15 soil depth (Table 2). The MA-C varied from 6.36 g kg–1 under control to 9.46 g kg–1 under T6 (75% NPK + 25% N- WS). Micro-aggregate associated carbon (mA-C) varied from 2.45 g kg–1 under control to 5.55 g kg–1 under T6 (75% NPK + 25% N-WS). Treatment T6 (75% NPK + 25% N- WS) increased 48.7 and 126% MA-C and mA-C respectively over control. Substitution of N from 25 to 50% by FYM or GM significantly increased MA-C and mA-C while WS significantly decreased MA-C and mA-C. The SOC concentrations were more in macro-aggregates (MA-C) than the micro-aggregates in all the treatments. Higher accumulation of C in macro-aggregates could be due to the more biophysical and chemical protection than in micro-aggregates (Ghosh *et al*., 2017) [6].

Table 2: Long term effect of manuring and fertilization on SOC associated with macro-aggregates (MA-C) and micro-aggregates (mA-C) in 0-15 cm depth under rice-wheat cropping system in Inceptisol

Treatments	MA-C (g kg^{-1})	mA-C (g kg^{-1})
Control (T_1)	6.36^g	2.45^g
100% NPK (T_2)	8.09^e	4.18^e
50% NPK + 50% N- FYM (T_3)	7.90^f	3.99^f
75% NPK + 25% N- FYM (T_4)	8.31^d	4.40^d
50% NPK + 50% N- WS (T_5)	8.09^e	4.18^e
75% NPK + 25% N- WS (T_6)	9.46^a	5.55^a
50% NPK + 50% N- GM (T_7)	8.96^b	5.05^b
75% NPK + 25% N- GM (T_8)	8.43^c	4.52^c

The data followed by different lower case letters in a particular column are significant according to Duncan's Multiple Range Test at P=0.05.

Glomalin associated with aggregates

Glomalin associated with macro-aggregates (GMA) and micro-aggregates (GMI) varied significantly across various treatments in 0-15 cm soil depth (Table 3). The GMA varied from 2.50 mg g^{-1} under control to 3.50 mg g^{-1} under T_7 (50% NPK + 50% N- GM). The GMI varied from 2.0 mg g^{-1} under control to 3.12 mg g^{-1} under T_3 (50% NPK + 50% N- FYM). The GMA was higher than GmA in all the treatments indicates that fungal protein plays an important role in the formation of macro-aggregates. MWD is strongly correlated with GMA (r=0.74*) and GmA (r=0.95**) (Table 4). Positive role of glomalin in the formation of WSMA was indicated by correlation coefficient.

Table 3: Long term effect of manuring and fertilization on glomalin associated with macro-aggregates (GMA) and micro-aggregates (GmA) in 0-15 cm depth under rice-wheat cropping system in Inceptisol

Treatments	GMA (mg g^{-1})	GmA(mg g^{-1})
Control (T_1)	2.50^e	2.00^g
100% NPK (T_2)	2.78^d	2.25^f
50% NPK + 50% N- FYM (T_3)	3.30^{bc}	3.12^a
75% NPK + 25% N- FYM (T_4)	3.34^b	2.59^d
50% NPK + 50% N- WS (T_5)	3.37^b	2.92^b
75% NPK + 25% N- WS (T_6)	3.18^c	2.39^e
50% NPK + 50% N- GM (T_7)	3.50^a	2.74^c
75% NPK + 25% N- GM (T_8)	2.90^d	2.52^d

The data followed by different lower case letters in a particular column are significant according to Duncan's Multiple Range Test at P=0.05.

Table 4: Correlation between MWD, WSMA, GMA and GmA under rice-wheat cropping system in Inceptisol

Parameters	MWD	WSMA	GMA	GmA
MWD	1			
WSMA	0.99**	1		
GMA	0.74*	0.73*	1	
GmA	0.95**	0.93**	0.82*	1

**. Correlation is significant at the 0.01 level (2-tailed) *. Correlation is significant at the 0.05 level (2-tailed). MWD-mean weight diameter, WSMA-water stable macro-aggregates, GMA-glomalin associated with macro-aggregates, GmA-glomalin associated with micro-aggregates.

Conclusions

The study clearly indicated that long-term application of balanced fertilizer alone or in conjunction with FYM, WS or GM favoured the soil aggregation and C buildup in the soil. Highest % WSMA (50.49%) and MWD (0.85 mm) was resulted under T3 (50% NPK + 50% N-FYM) while aggregate associated C increased by 48.7% (MA-C) and 126% (mA-C) respectively under T6 (75 % NPK + 25 % N-WS) over control. Glomalin content was positively related to the stability of aggregates as indicated by the strong correlation of MWD with GMA (r=0.74*) and GmA (r=0.95**).

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