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Application of novel organically derived fulvic acids in soil aggregate size distribution, fraction, organic carbon, and nitrogen content in three distinct soils

 Mahendar Kumar Sootahar,¹ ^{ab} Abdul Ghafoor,^{2,c} Mukesh Kumar Sootahar,⁹ Muhammad Munir,^e Hassan Ali-Dinar,^e Zafar Iqbal,^f Kashif Ali Kubar ^d and Hafeezullah Babar^a

The application of organic manure/substances in soil can directly alter soil structure and influence the soil organic carbon (SOC), soil aggregate stability, and aggregate-associated nitrogen (N). In this study, a pot experiment was conducted to evaluate the influence of three organically derived fulvic acid (FA) treatments—fulvic acid powder (S, 0.25%), natural liquid (NL, 0.50%) and plant-derived liquid (P, 0.50%)—alongside a control (CK) on aggregate size distribution (>250 μm, 250–53 μm and <53 μm sizes), SOC, total nitrogen (TN), water-stable aggregates, and mean weight diameter (MWD) of aggregates in three typical soils: albic black (AL), irrigated desert (IR) and Shahjiang black (SH). The results showed that water-stable aggregates increased by 39.6% when using 0.25% S amendment in AL soil compared with the control, while the maximum MWD (1.51) was obtained with 0.50% P in AL soil. The SOC and TN contents of >2 mm aggregates were significantly enhanced in IR soil (35–40% and 60–80%, respectively), whereas 2–0.25 mm fractions exhibited higher SOC and TN in AL soil compared with the other soils. Furthermore, SOC and TN distribution in the 0.25–0.053 mm aggregate fraction increased by 12–40% and 12–16%, respectively, with SH soil. Microaggregates (<0.053 mm) exhibited the highest TN in AL and the highest SOC in SH soil. This study demonstrates that fulvic acid application, particularly in liquid or solid forms, substantially improves soil aggregation, enhances SOC and N sequestration in different aggregate fractions, and contributes to better soil health. These findings provide new evidence supporting the use of fulvic acids as an effective amendment for improving soil structure and nutrient retention, offering a sustainable strategy for soil quality restoration and long-term agricultural productivity.

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1. Introduction

Soil organic carbon represents the most substantial reservoir within the global terrestrial carbon cycle, with an estimated 1500 petagrams of carbon.^{1,2} This amount surpasses the amount of carbon stored in vegetation and the atmosphere combined.³ Owing to its significant proportion, any increase in

SOC concentration can lead to a substantial rise in the release of carbon dioxide (CO₂) into the atmosphere, contributing to global warming.⁴ The breakdown of SOC is an essential process connected to nutrient release, soil nutrient enhancement, emission of greenhouse gases, and agricultural productivity.⁵ Soil aggregates perform a vital role in preserving soil fertility as well as soil structure by providing physical protection of SOC, thus preventing its mineralization. Many consider the stability of soil aggregates to be a crucial sign of the evolution of soil structure, deterioration, and stabilization.^{6,7} Concerns have been raised over the increased use of both organic and artificial fertilizers in agriculture in recent decades, due to the potential for soil structural degradation and reduced efficiency in resource utilization.^{8,9} Therefore, it is highly recommended to use the best fertilization techniques to increase agronomic productivity, soil quality, and soil carbon sequestration.^{10,11}

The stability of soil aggregates and their associated carbon serves as a crucial indicator of overall soil health, contributing to enhanced functions within agroecosystems.^{12,13} Soil aggregates protect SOC from disturbance by microbes by enclosing it within various aggregate size fractions.^{14,15} The stability of

^aSoil Fertility Research Institute, Agriculture Research Center, Tando Jam, 70060, Pakistan. E-mail: mahender_935@yahoo.com; hafeezbabartj@gmail.com

^bInstitute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agriculture Sciences, Beijing 100081, China

^cCenter for Water and Environmental Studies, King Faisal University, Al-Ahsa 31982, Saudi Arabia. E-mail: aghafoor@kfu.edu.sa

^dLasbela University of Agriculture Water and Marine Sciences, Uthal, Pakistan. E-mail: kashifkubar@luawms.edu.pk

^eDate Palm Research Center of Excellence, King Faisal University, Al-Ahsa 31982, Saudi Arabia. E-mail: mmunir@kfu.edu.sa; hdinar@kfu.edu.sa

^fCentral Laboratories, King Faisal University, PO Box 420, 31982 Al-Ahsa, Saudi Arabia. E-mail: Mnsattar@kfu.edu.sa; zafar@kfu.edu.sa

⁹College of Natural Resources and Environment, Northwest A&F University, Yangling, 712100, China. E-mail: mukeshksootar@gmail.com



mineral organic matter is related to the protection of soil nutrients and associated carbon may be useful to microbiota sites.¹⁶ The efficacy of aggregates in safeguarding SOC is size-dependent, with macroaggregates (>250 μm) demonstrating lower efficiency compared to microaggregates.^{6,16} To improve soil aggregation, it is essential to comprehend how land management techniques, including fertilization, affect the distribution of aggregate sizes and the corresponding quantities of nitrogen (N) and SOC. Numerous studies have established a direct correlation between SOC levels and the application of appropriate quantities of organic amendments, including in farmyards (FYM), crop remains, and straw yields.^{17,18}

Soil organic carbon makes up the largest single reservoir in the world,¹⁹ and given its substantial proportion, this may lead to a significant rise in atmospheric carbon dioxide (CO_2) release, potentially contributing to global warming.²⁰ In addition to affecting greenhouse gas emissions and food production, the mineralization of SOC plays an essential role in nutrient release, soil nutrient dynamics, and quality enhancement.⁵ One important measure of soil fertility is SOC, and differences in SOC levels cause changes in soil aggregation.²¹ Soil aggregates are essential for preserving soil fertility and structure by providing physical protection to SOC, which is protected from mineralization by soil aggregates, which act as a barrier. Moreover, the stability of these aggregates is usually recognized as a crucial indicator of the processes involved in the formation, breakdown, and maintenance of soil structure.^{7,15} Concerns regarding the possibility of soil structural degradation have been raised in recent decades, as a result of the growing use of both organic and inorganic fertilizers in agriculture.^{8,9} Consequently, the implementation of optimal fertilization practices is strongly recommended to enhance soil quality, improve soil carbon sequestration, and increase agricultural performance.^{10,11} Likewise, in evaluating the health of soil, one important metric is the stability of soil aggregates and the amount of carbon (C) they contain, which is vital for improving the functions of agroecosystems.^{12,13} The capacity of aggregates to protect encapsulated SOC varies with their size, as macroaggregates (>250 μm) are generally less effective than microaggregates.²² Thus, to optimize soil aggregation, it is crucial to comprehend how land management practices, including fertilization, influence aggregate size distribution as well as the associated SOC and nitrogen (N) content. Extensive research has shown that increasing SOC levels is closely related to the application of suitable quantities of organic amendments, including manure, crop residues, and straw.^{17,22,23} Moreover, the depletion of SOC in a given soil is influenced by both its SOC content and the degree of soil aggregation.^{24,25} In other words, carbon sequestration can be encouraged by appropriately managing agricultural soils, such as by using organic manure and preserving crop waste.^{26,27}

The addition of organic substances and manure can directly change the aggregation and stability of aggregates,^{28,29} and C and N linked with aggregates can also be changed.^{30,31} Many researchers suggested that tillage practices and other conventional methods increase the decomposition of SOM,^{29,32,33} and

decrease the SOM content³⁴ in macroaggregates.³⁵ Thus, these practices inhibit the formation and stabilization of macroaggregates, and also decrease the physical protection of SOM with macroaggregates, increasing the C and N content linked with macroaggregate.^{30,35,36} The use of organic manures can also reduce the soil layer development and reduce the water surplus. It has also been reported that the addition of organic manure as a nutrient source can shelter C and N during the accretion of macroaggregates.³⁷ Yu *et al.*³⁸ evaluated the long-term potential of organic manure and mineral fertilizers on aggregation and aggregate-associated carbon in a sandy loam soil. Li *et al.*³⁹ assessed the long-term influence of manure and inorganic fertilizers on soil organic carbon and its labile fractions in bulk soil. Gautam *et al.*⁴⁰ examined the impact of manure and inorganic fertilization on aggregate stability, soil nutrients, organic carbon and nitrogen in different aggregate fractions in soil. Liu *et al.*⁴¹ assessed the potential of straw return practices on soil aggregates and organic matter concentration within the farmlands of the Northeast Black Soil Region. Based on the above facts and our current understanding of the effects of organic substances and manures, we hypothesized that the distribution of organic carbon and nitrogen across aggregate fractions and the stability of aggregates in different soil types remain largely unknown. However, the effect of organic substances and manure on soil quality and soil structure has not been documented on a pot experiment level. Therefore, this study was designed to systematically investigate the effect of three fulvic acid sources—powder, natural liquid and plant-derived liquid—on aggregate stability, SOC, TN and aggregate size (>250 μm , 250–53 μm and <53 μm) in three distinct soil types (albic black, irrigated desert and Shahjiang black). This research provides new insights into how FA application can enhance soil aggregation and nutrient retention, offering a sustainable strategy for soil health restoration and carbon management.

2. Materials and methods

Soil samples were collected from Qiqihar and Wuwei, as well as from the experimental station of Anhui Agricultural University, situated in the Heilongjiang, Gansu, and Anhui provinces of China (Fig. 1). Soils of different types (Luvisol, Inceptisol and Vertisol) are used to investigate how the application of organic substances and the characteristics of organic manure influence aggregate size distribution, soil organic carbon (SOC), total soil nitrogen (TSN), and the carbon and nitrogen content associated with soil aggregates in albic black soils (AL), irrigated desert soils (IR), and Shahjiang black soils (SH). Sample collecting sites were in subtropical regions, having 15 °C annual mean temperature, and the precipitation was varied. The AL soil was used for planting corn on around 584 farms of that region. The IR soil was from newly reclaimed land, which is likely difficult for crop cultivation.⁴² The SH soil is a grain production soil with annual production of cash crops, such as maize, rice and wheat, and rotation crops have been cultivated for the last 2 decades. All these soils were managed by conventional tillage and the same type of management practice was applied as those mostly used in Chinese cultural practices. Soil megaliths with 10 cm diameters



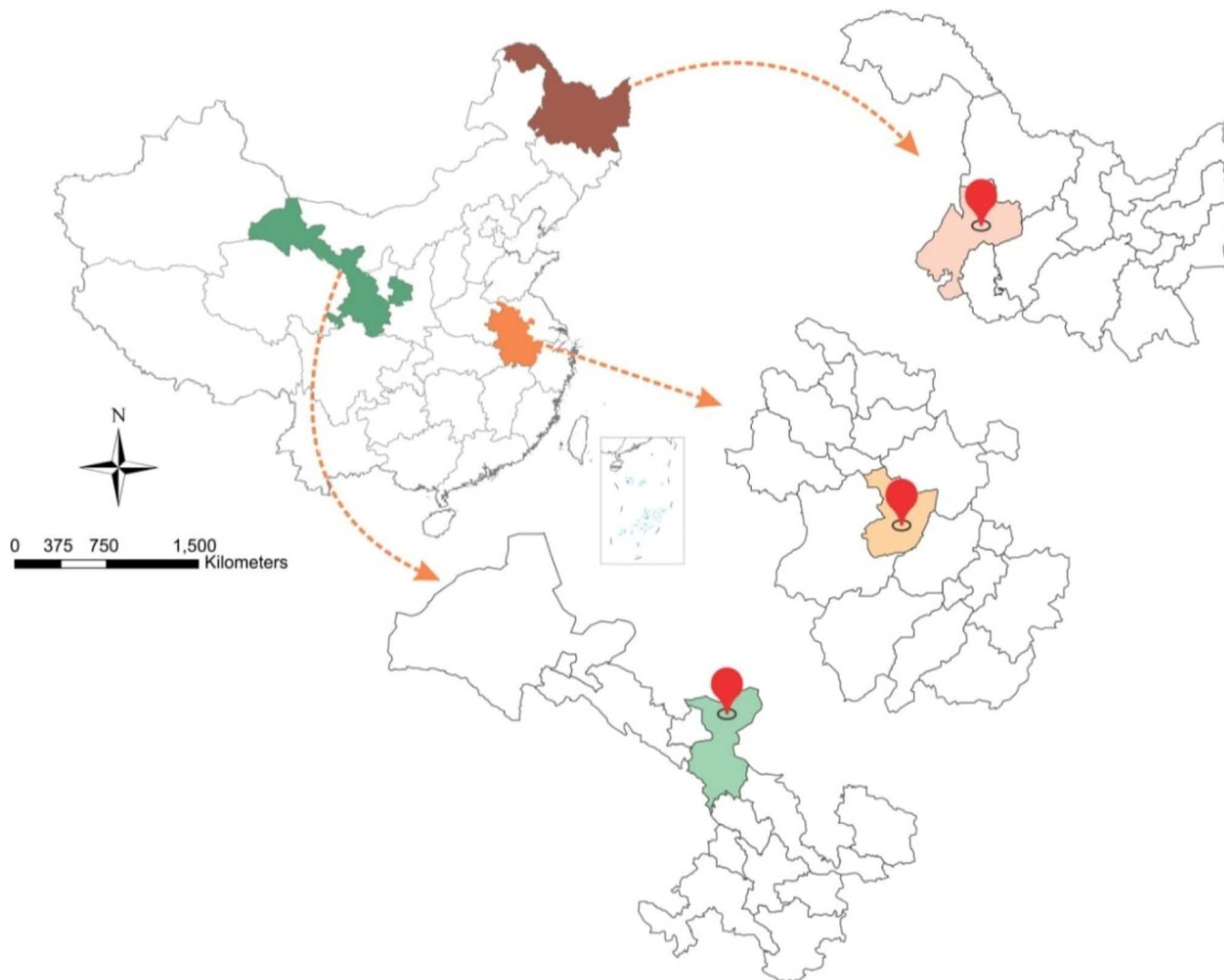


Fig. 1 Image representing the sample collection locations in three different provinces of China: Heilongjiang, Gansu and Anhui provinces.⁴²

were randomly sampled from each site at a depth of 10 cm below the soil surface. The soil samples were transported to the soil remediation laboratory with their initial structures, air-dried, and broken into smaller parts along natural cracks by hand.

2.1. Experimental design

The pot experiment comprised four treatments: 0.25% fulvic acid powder (S), 0.50% natural liquid (NL), and 0.50% plant liquid (P) fulvic acid (FA), with an unamended control group.⁴³ This was arranged in a randomized complete block design with three replicates for each treatment across the different soils, along with a measurement plot of 50 × 60 cm in area. Fulvic acids were extracted using the method established by the International Humic Substances Society (IHSS) and obtained from Fertilizer Co. Ltd (ISS 2022). The pots of 27 and 29 cm in height and diameter were filled with 15 kg of air-dried soil sieved with a 5 mm sieve. To maintain an equal surface level, pots were buried in the soil, and 5 to 10 wheat seeds were planted in each pot along with initial compound fertilizer amounts of 25%, 14% and 7% N, P₂O₅ and K₂O, respectively. FA in the form of liquid

and solid were applied to each pot: the liquid FA was applied with water, while the solid FA was incorporated into the soil.

2.2. Water-stable aggregates

Water-stable aggregates from different size fractions were obtained for each sample from different sites. Approximately 50 g of the dried sample was placed on the top of the sieve having net sizes of 2 mm, 1 mm, 0.25 mm and 0.053 mm, from highest to lowest. A set of sieves was placed on a rack for aggregate Yoder shaker analysis (Institute of Regional Planning and sustainable graduate of Chinese academy of agricultural sciences, Beijing China), and the samples were added to water at a frequency of 30 times per minute for a duration of 30 min. Sieves of each fraction size were washed into an empty beaker marked up with the initial weight of the empty beaker, left to dry and weighed.

The mean weight diameter (MWD) was calculated as follows:

$$\text{MWD} = \frac{\sum 4i = 1d_i W_i}{W}$$



Table 1 Elemental composition of solid (S), natural liquid (NL) and plant-derived liquid (P) FA

FA type	%			
	N	C	H	S
Solid (S)	5.39	25.31	5.75	8.47
Natural	10.29	52.476	9.74	14.84
Plant-derived	10.78	50.61	11.56	16.96

where d_i is the average diameter of the i^{th} size fraction of the aggregates, W_i is the mass of the i^{th} size fraction of the aggregate, and W is the total mass of all size fractions of the aggregate. $i = 1, 2, 3$ and 4 represent aggregate sizes of >2 mm, $2-0.25$ mm, $0.25-0.053$ mm and <0.053 mm, respectively.

2.2.1 Elemental composition of fulvic acids (FA). Shandong Quan Linjia Fertilizer Co. Ltd (Shandong, China) provided the soil-applied fulvic acids, and the chemical compositions of three different FA are presented in Table 1.

2.3. Statistical analysis

The means \pm standard error (SE) are used to display the data. At a significance threshold of $p < 0.05$, means ($n = 4$) were compared using Bonferroni multiple comparison tests and one-way analysis of variance (ANOVA). The Statistical Package for the Social Sciences (SPSS, Version 21.0, Chicago, IL, USA) was used for statistical analyses, and GraphPad Prism 6 was used to plot the graphs.

3. Results

The fundamental soil properties, including pH, electrical conductivity, soil organic matter, TN, total phosphorus (TP), and total potassium (TK) contents for the three study locations are provided in Table 2. Each soil developed from different parent materials, resulting in significantly different physico-chemical properties. The AL soil exhibited the lowest electrical conductivity ($30 \mu\text{S cm}^{-1}$) and pH (5.26). Conversely, the IR soil demonstrated the lowest concentrations of organic matter (OM), cation exchange capacity (CEC), and TN, when compared to AL and SH soils (Table 2). Additionally, Table 2 indicates that SH soil exhibited the highest levels of organic matter, CEC, and TN content. Additional soil characteristics, such as TP and TK contents, did not significantly differ among the three soils. However, due to differences in the parent material, the silt, sand, and clay contents also exhibited variability. The AL soil displayed a higher proportion of silt particles, while the IR soil had a sandy texture. In contrast, the SH soil exhibited stickiness when wet, attributed to its elevated clay content.⁴⁴

3.1. Aggregate size distribution and mean weight diameter of aggregates

Treatment with fulvic acid significantly influenced the aggregate size distribution and mean weight diameter of the soil aggregates (Table 3). Contents of water-stable aggregates of AL, SH and IR soils ranged between 37.7% and 39.6%, 36.2% and 37.4%, and 38.1% and 39.0%, respectively (Table 3). Fulvic acid

Table 2 Chemical properties of the soil used in this experiment

Soil	Coordinates	EC ($\mu\text{S cm}^{-1}$)	pH	OM (g kg^{-1})	CEC (cmol kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	Textural class
Albic	47° 21' N, 123° 55' E	30.1	5.26	8.4	21.6	0.70	0.39	20.4	Silty clay
Irrigated	37° 55' N, 102° 38' E	2063	8.49	2.2	4.6	0.20	0.39	20.1	Sandy loam
Shahjiang	33° 50' N, 117° 16' E	132	7.99	11.7	25.2	0.83	0.39	17.3	Clay loam

Table 3 Mass distribution between aggregates (g), WSA (g) and MWD (mm)

Soil	Treatment	Aggregate fractions (mm)				Total WSA	MWD mm
		>2	$2-0.25$	$0.25-0.053$	<0.053		
AL	Control	2.29bc	7.20bc	7.70b	20.60 ab	37.8	1.36
	0.25% S	3.32bc	6.89bc	8.32b	21.07a	39.6	1.38
	0.50% NL	3.12bc	6.61bc	8.10b	20.24 ab	38.0	1.38
	0.50% P	2.24bc	8.45b	9.16b	17.90b	37.7	1.51
IR	Control	1.35c	4.32c	23.20a	10.0c	38.9	0.85
	0.25% S	0.84c	5.77bc	22.52a	9.92c	39.0	0.96
	0.50% NL	1.32c	4.32bc	24.23a	8.90c	38.7	0.84
	0.50% P	0.54c	4.40bc	24.19a	9.03c	38.1	0.77
SH	Control	8.42a	22.69a	3.63c	2.64d	37.3	4.18
	0.25% S	6.33 ab	23.42 ab	4.81c	2.91d	37.4	4.02
	0.50% NL	9.53a	20.86a	3.44c	2.37d	36.2	4.22
	0.50% P	7.84a	23.87a	3.67c	2.08d	37.4	1.23

The letters a, b, c and d are used to indicate the significance level of results between the aggregates in different soils and also between aggregates under different treatments, where a is the highest significance and d the lowest.



powder (0.25% S) treatment significantly increased the water-stable aggregates (WSA) as compared to other treatments. Irrespective of the treatments, the percentage of macro and microaggregates exhibited a significant difference between the soils, and the microaggregate <0.053 mm fraction showed the greatest percentage of 17.9–21.0% for the AL soil as compared to macroaggregates. However, the >2 mm fraction presented the lowest percentage of 2.2–3.3% as compared with other soils for the treatments. Meanwhile, the 0.25–0.053 mm fraction exhibited the greatest contribution of 22.5–24.2% for the IR soil, and the lowest contribution of 0.54–1.32% was detected for the >2 mm fraction. The aggregate size distribution of the SH soils varied a lot compared to the other two soils. The highest aggregate proportion of 20.8–23.8% was observed for the 2–0.25 mm fraction, and the lowest proportion was 2.0–2.9%, determined for the <0.053 mm fraction for SH soil. The mean weight diameter (MWD) values were non-significantly higher after the application of fulvic acid (FA) treatment than those for the control treatment in the three soils (Table 3). The highest MWD values (1.51, 0.96 and 4.22 mm) were noted with the plant-derived fulvic acid (0.50% P) treatments as compared to the control (1.36, 0.77 and 3.23 mm) for the AL, IR and SH soils, respectively.

3.2. Distribution of SOC and total nitrogen (TN) content in the >2 mm macroaggregate fraction

The effect of fulvic acid (FA) on SOC and TN distributions in the aggregates in the three soils in the >2 mm fraction was large for the SOC distribution. The overall results revealed that the

distribution of SOC in the three soils with the treatments showed the highest value in the >2 mm macroaggregate fractions as compared with other microaggregate fractions (Fig. 2). The highest SOC contents for the >2 mm aggregates were observed in the IR soil and were significantly increased by 35–40% under 0.50% P FA treatment. However, the lowest contents were observed for AL soil and were significantly decreased by 19–25% as compared to other soils. Correspondingly, the TN contents of the >2 mm aggregates were observed to be the highest for the IR soil and were significantly increased by 60–80%. The lowest contents were observed for the SH soil and were significantly increased by 30–40% as compared to other soils.

3.3. Distribution of SOC and TN in the 2–0.25 mm aggregate fractions

The effect of FA treatment on the SOC and TN distributions in the aggregates for the three soils in the 2–0.25 mm aggregate fractions was large for the SOC distribution. The data show that the distributions of SOC for the 2–0.25 mm aggregate fractions were highest in AL and SH soils as compared with other soils under 0.50% P FA treatment (Fig. 3). The highest SOC contents of the 2–0.25 mm aggregates were observed for the AL soil and were non-significantly increased by 14–18%, and the lowest were detected for the IR soil and were significantly increased by 40–44%. However, the content of the SH soil was non-significantly decreased by 10–12%, as compared with the other soils. Correspondingly, TN contents were observed to be the highest for the AL soil, and were non-significantly decreased

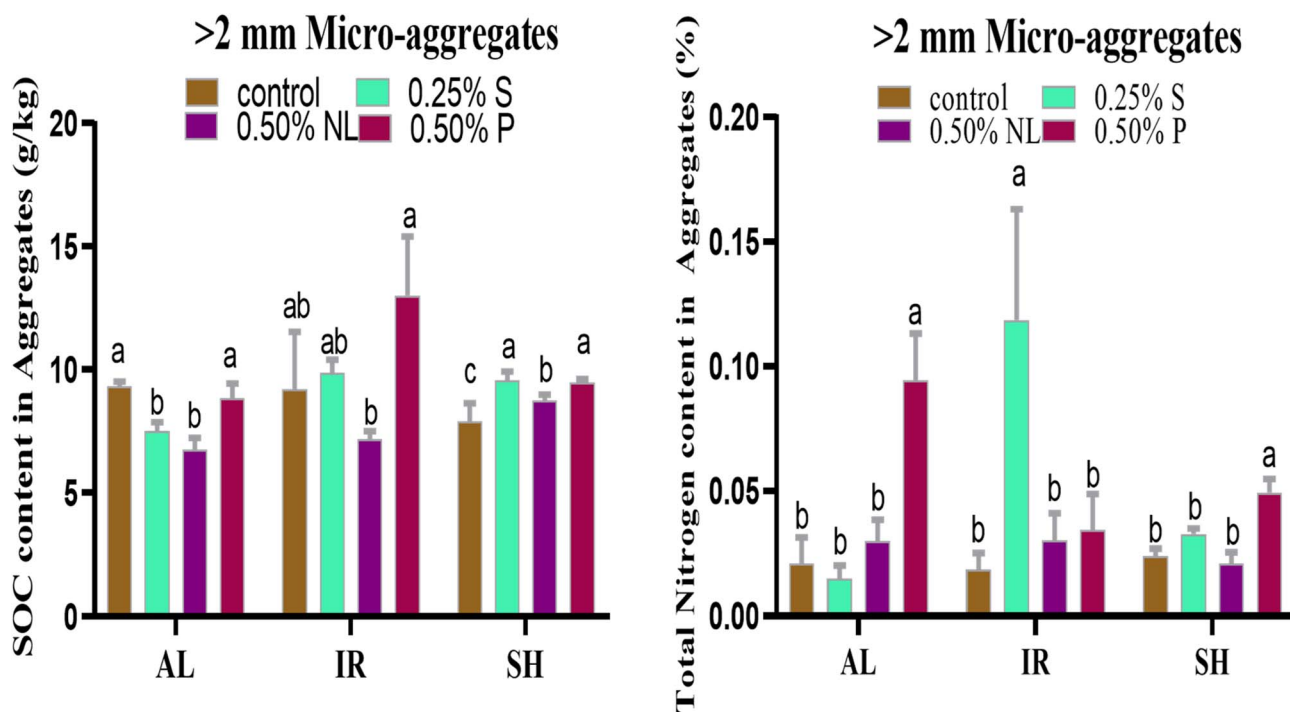


Fig. 2 Distribution of SOC and TN content in >2 mm macroaggregate fractions after the application of S, NL and P FA to AL, IR and SH soils. Standard errors of the mean ($n = 4$) are shown by error bars. At the 5% level of significance, the least significant difference (LSD 0.05) is presented. The letters above each bar (a, b, and c) indicate a substantial difference between the treatments.



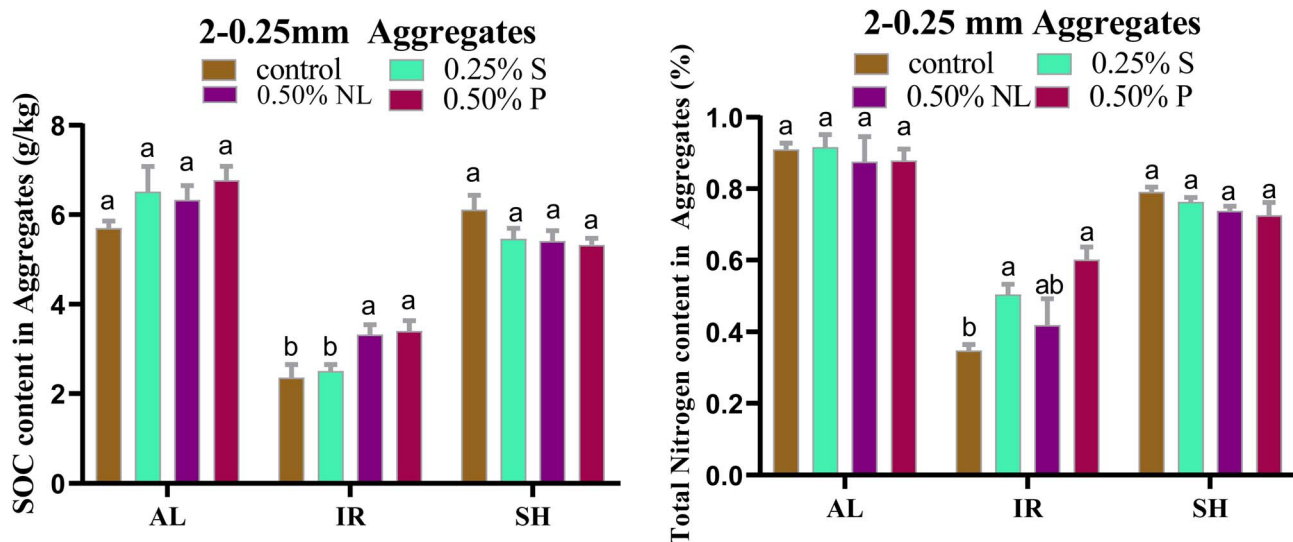


Fig. 3 Distribution of SOC and TN content in the 2–0.25 mm aggregate fraction after the application of S, NL and P FA to AL, IR and SH soils. Standard errors of the mean ($n = 4$) are shown by error bars. At the 5% level of significance, the least significant difference (LSD 0.05) is presented. The letters above each bar (a, b, and c) indicate a substantial difference between the treatments.

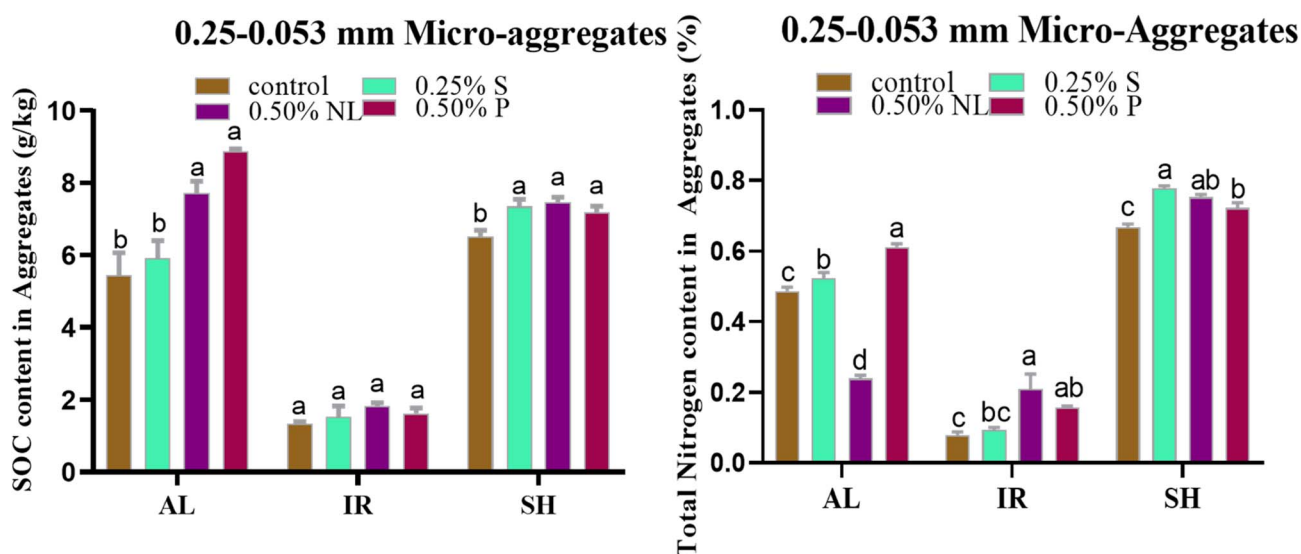


Fig. 4 Distribution of SOC and TN content in the 0.25–0.053 mm aggregate fraction after the application of S, NL and P FA to AL, IR and SH soils. Standard errors of the mean ($n = 4$) are shown by error bars. At the 5% level of significance, the least significant difference (LSD 0.05) is presented. The letters above each bar (a, b, and c) indicate a substantial difference between the treatments.

by 3–4%, and the lowest contents were detected for the IR soil and were significantly increased by 50–60% as compared to the other soils. The contents of the SH soil were non-significantly decreased by 6–8% for the treatments.

3.4. Distribution of SOC and TN in the 0.25–0.053 mm microaggregate fraction

The effect of FA on SOC and TN distributions in the aggregates in the three soils in the 0.25–0.053 mm aggregate fractions was large for the SOC distribution. The results show that the

distributions of SOC in the AL and SH soils were observed to be the highest in the 0.25–0.053 mm microaggregate fractions, as compared with the IR soil with 0.50% P FA treatment (Fig. 4). The highest SOC contents of the 0.25–0.053 mm micro-aggregates were observed for SH, followed by the AL soil, and were significantly increased by 12–40% and 40–60% under 0.50% P FA treatment, and the lowest contents were detected for the IR soil and were non-significantly increased by 20–30% as compared to other soils. Likewise, the TN contents were observed to be the highest for the SH soil and significantly



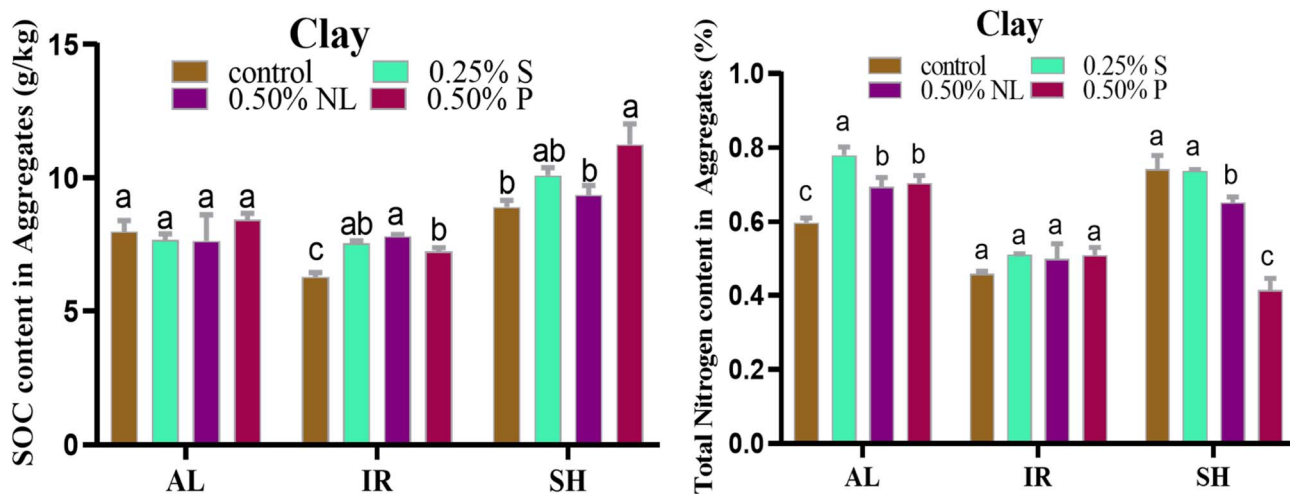


Fig. 5 Distribution of SOC and TN content in the <0.053 mm aggregate fraction after the application of S, NL and P FA to AL, IR and SH soils. Standard errors of the mean ($n = 4$) are shown by error bars. At the 5% level of significance, the least significant difference (LSD 0.05) is presented. The letters above each bar (a, b, and c) indicate a substantial difference between the treatments.

increased by 12–16% under 0.50% P FA treatment, and the lowest were detected for the IR soil, and were significantly increased by 70–80% as compared with the other soils. However, in the AL soil the content was significantly increased by 20–25% for the treatments.

3.5. Distribution of SOC and TN in the <0.053 aggregate fractions

The effect of FA on SOC and TN distributions in the <0.053 mm fractions in the three soils was large for the SOC distribution. The results showed that the highest distribution of SOC in the <0.053 mm microaggregate fractions was observed in the SH soil under 0.50% P FA treatment compared with the other soils (Fig. 5). The highest SOC contents in the <0.053 mm aggregates were observed for SH and were significantly increased by 12–16%, and the lowest were detected for the AL soil and were significantly increased by 4–5%. Similarly, TN contents were observed to be the highest for the AL soil and were significantly increased by 15–30% under 0.50% P FA treatment, and the lowest were detected for the IR soil and were significantly increased by 8–10%. However, TN contents of the SH soil were significantly decreased by 20–30% for the treatments.

4. Discussion

4.1. Aggregate stability

The mean weight diameter (MWD) and water-stable aggregates (WSA) are defined as the physical characteristics of a soil, which ultimately determine the fertility status of the soil. The application of fulvic acid (FA) helps in improving these two indicators. Water-stable aggregates (WSA) were recorded to be higher with 0.25% S FA treatment. This higher WSA might be due to the application and expression through the incorporation of a greater quantity of organic matter in the form of treated FA. It might also be because this type of FA was in the solid form and not dissolved completely, as compared with the other two FAs.

Another reason could be that organic C residue increases the availability of microorganisms and helps to enhance the soil fertility, and also plays an important role in aggregate binding. Comparatively, less difference was found in WSA values between the soils with the treatments. However, a greater amount of WSA for the AL soil compared to the other two soils might be attributed to the accrual of organic carbon and the development of organic–inorganic complexes. The occurrence of a higher proportion of aggregates in AL was due to the change from microaggregate-associated C and N to macroaggregate-associated C and N. However, the proportions of macroaggregates in the IR and SH soils were determined to be higher as compared with microaggregates, which could be because the soils originated from various parent materials and had different soil textures. Another reason might be that after harvesting, the maize soils were tilled and particles were not exposed. These findings align with the work in ref. 45, in which it was noted that microaggregates are more stable than macroaggregates, so they require a greater force to disrupt during tillage.^{46,47} Our results are in agreement with studies conducted earlier.^{45,48} Puget *et al.*⁴⁹ also observed that tillage resulted in a decreased amount of WSA. However, the observation in our research study suggests that, the pots were not tilled and only hand hoeing occurred when soils were transferred into pots after the maize was harvested, and cultivated wheat was seeded the next year. Thus, macroaggregates were disrupted and microaggregates were formed. In addition with only few sieves, the slaking techniques eliminate the macroaggregates of relatively low firmness, and only highly firm aggregates will remain. Relatively, a lower proportion of macroaggregates at AL soil compared to the IR and SH soils among the treatments was observed, when the crop was cultivated and was attributed to defiance into microaggregates due to because soil was very hard to compact and came in contact with macroaggregates. However, the aggregate proportions of the IR soil were detected to be higher for 0.25–0.053 mm, which is due to the IR being



a sandy loam soil and easy to hoe, and likewise, the SH soil had a high aggregate proportion for 2–0.25 mm, which might be because the SH soil was hard when dry and moist when wet. Another reason could be that the aggregates were determined on dry soil. The aggregate proportion could exhibit similar results if the material was applied for long-term experiments and in the field; however, time was too limited to determine these aggregate proportions. Macroaggregates exhibit lower stability compared to microaggregates; consequently, they are more prone to the disruptive forces associated with various tillage practices.⁴⁶ The proportions of WSA in the macroaggregates in the three soils with the treatments were observed to be higher, but there was not much difference between the soils. This could be ascribed to the fact that many microbial organisms decomposed the different organic products and sticky elements, created microaggregates from soil particles and combined them to form macroaggregates. The fulvic acid acts as a labile carbon source, stimulating microbial growth and activity, including microbes that produce extracellular polymeric substances (EPS), which help glue soil particles and aggregates together. Tang *et al.*⁵⁰ (2021) demonstrated that the application of fulvic/humic substances increased dissolved organic matter and microbial enzyme activity, and in turn, enhanced carbon sequestration in black soils. Moreover, recent studies showed that FA can enhance extracellular electron transfer (EET) in soils more efficiently than humic acids through functional groups such as carboxyl and phenolic groups, which can facilitate redox reactions and possibly affect aggregation by mediating mineral–organic interactions and stabilizing mineral surfaces.⁵¹ Singh *et al.*⁵² proved that the addition of inorganic fertilizers along with organic manure in a rice–wheat system increased the proportion of soil aggregates and improved soil fertility. Similarly, Benbi *et al.*⁵³ found that the 20 year application of farmyard manure on a maize–wheat–cowpea cropping system significantly increased the total water-stable aggregates (WSA) by 12% compared to the unfertilized control. They also reported that farmyard manure application increases the WSA in all size fractions (>2 mm, 1–2 mm, 0.5–1.0 mm, 0.25–0.50 mm and 0.11–0.25 mm). Yang *et al.*⁵⁴ suggested that the application of farmyard manure, and wheat straw significantly increases the WSA in the >0.25 mm fraction size.

4.2. Distribution of carbon and nitrogen associated with aggregates

According to the results of our study, the C densities of the macroaggregates were demonstrated to be higher as compared with microaggregates for the treatments among all three soils. The macroaggregate size fraction of >2 mm had the highest C density, and it was reduced when the aggregates became finer. The results also showed that the C density accretion increases when FA is applied at 0.50% P in different aggregates for the soils as compared with other treatments. Organic C is formed by the binding of different types of organic compound in between the macroaggregates and is physically attached within the macroaggregates. The stimulation and addition of C in the aggregates were cemented by different agents and are described by the

humification of organic matter. The mean aggregate C was higher in AL and SH soil as compared to IR soil in the treatments. This may be due to the IR soil's sandy texture, characterized by coarse soil particles that are very difficult to bind into aggregates. However, AL and SH soil are silt clay and clay loam in nature, respectively, and the formation of these soil aggregates is much easier, allowing them to retain the C and N within the aggregate fractions. These findings are consistent with the results of Puget,⁴⁹ who observed that the accumulation of C was greater in macroaggregates, and that this was because the organic matter was slowly decomposed, related to these aggregates, and who also observed that organic matter directly enhances the stability and C in macroaggregates. Aoyama *et al.*³⁷ observed that mineral-associated organic matter in aggregate fractions is increased after the manure application. They suggested that mineral-associated organic matter, which originates from the decomposition of particulate organic matter, facilitates the formation and stabilization of macroaggregates. Cambardella and Elliott observed a greater quantity of total carbon in macroaggregates compared to microaggregates. According to their study, inorganic fertilizer application alone or in combination with compost increases the C sequestration in macroaggregates. The quantity of C accrued in the >2 mm fraction was recorded as higher, and it decreased when the aggregate fraction was decreased.

The amount of total N density in macroaggregates was observed to be higher than in microaggregates. The total N and C density can be stabilized more easily in macroaggregate fractions. According to our results, it was observed that the macroaggregates contained a higher N density than the microaggregates. This could be because FA and other organo-mineral compounds play important roles in mixing and binding these aggregates, such as microaggregates into macroaggregates, and through this, the N stability and its content were also increased. This could be attributed to insufficient microbial decomposition to break down the total N content in FA within macroaggregates, resulting in higher total N content. Mikha and Rice⁵⁵ observed that N is significantly increased in aggregates after the application of organic manure. Aoyama *et al.*³⁷ also found that N in macroaggregates is protected after organic manure application. They also found that the total N content was increased in the 0.25–1.0 mm fractions of aggregates as compared with other fractions. Soil C and total N density after the application of FA in all three soils were increased in macroaggregates and decreased as the aggregate size decreased. The results are consistent with the findings of Dick and Gregorich,^{56,57} who reported that manure and raw materials, after application to soil, resulted in decomposition and the formation of organic substances, and these substances converted organic C into recalcitrant C, thus a high amount of C was sequestered into the soil. Manna *et al.*⁵⁸ identified that an unfertilized plot decreased the soil organic C content by 15.5–41.5% as compared to the initial value at three sites, and in the same way, the application of inorganic fertilizers, NPK and NPK + FYM treatments, increased or sustained the C content compared to the initial values for the three soils.

The application of 0.50% P FA increased the total N in the three soils in all the aggregate fractions. The highest content



of N was recorded for the 2–0.25 mm aggregates for the treatments. After the application of FA there was an increase in the total N in the aggregates, showing the capability of the soil to hold enough N for better crop production and a higher yield. Gautam *et al.*⁴⁰ suggested that the application of organic manure in aggregates resulted in a higher N content than the application of inorganic or no N fertilizers. These findings have also been reported by many other researchers,^{36,54} who found that manure application had a significant effect on the increase of the total N content within the aggregates.

5. Conclusion

The application of solid (S) 0.25%, natural liquid (NL) 0.50% and plant liquid (P) 0.50% FA treatments significantly increased soil structure and fertility parameters, including aggregate stability, mean weight diameter and C sequestration in AL, IR and SH soils. FA treatments enhanced aggregate stability and MWD, and increased the C and N density in macroaggregates in the three soils, thereby improving soil resilience and long-term organic matter storage. The total N and C contents associated with the aggregates were observed to be higher in the macroaggregate fraction and decreased as the aggregate fractions decreased. Overall, whether applied in the liquid or solid state, FA provides a sustainable option for soil management, with the benefit of enhancing soil fertility. Given these promising results, future studies should explore FA integration with nanomaterials to further increase aggregate stability, elucidate the binding mechanism, and quantify its role in nutrient cycling, microbial activity, and long-term C stabilization for diverse soil types.

Conflicts of interest

The authors declares there is no conflict of interest.

Abbreviations

SOC	Soil organic carbon
TN	Total nitrogen
FA	Fulvic acid
C	Carbon
N	Nitrogen
AL	Albic black
IR	Irrigated desert
SH	Shahjiang black
S	Solid
NL	Natural liquid
P	Plant-derived
WSA	Water-stable aggregate
MWD	Mean weight diameter
OM	Organic matter
CEC	Cation exchange capacity
TP	Total phosphorus
TK	Total potassium
EC	Electrical conductivity

Data availability

The data associated with this manuscript can be made available upon request from the authors.

Supplementary information is available. See DOI: <https://doi.org/10.1039/d5ra04731e>.

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