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ORIGINAL

The Regulation of Soil Organic Carbon Sequestration in Calcareous Soil by Long-Term Organic Amendments and Nitrogen Fertilization

Regulación del secuestro de carbono orgánico del suelo en suelos calcáreos mediante enmiendas orgánicas a largo plazo y fertilización nitrogenada

Priyadarshani A. Patil[1], Banothu Vijay[2], Subhaprada Dash[3], Harsimrat Kandhari[4], Geetika M. Patel[5], Madhur Taneja[6]

[1]Krishna Institute of Science and Technology, Krishna Vishwa Vidyapeeth "Deemed to be University". Taluka-Karad, Dist-Satara, Pin-415 539, Maharashtra, India.
2Centre for Multidisciplinary Research, Anurag University. Hyderabad, Telangana, India.
[3]Department of Agronomy, Institute of Agricultural Sciences. Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India.
[4]Chitkara Centre for Research and Development, Chitkara University. Himachal Pradesh-174103 India.
[5]Department of Community Medicine, Parul University. PO Limda, Tal. Waghodia, District Vadodara, Gujarat, India.
[6]Centre of Research Impact and Outcome, Chitkara University. Rajpura- 140417, Punjab, India.

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Corresponding Author: Priyadarshani A. Patil

ABSTRACT

There is compelling evidence that organic inputs encourage soil health and sustainable agriculture. Nonetheless, it is currently unclear how long-lasting organic additions in combination with chemical fertilizers affect soil macro aggregate production and the soil's ability to store organic carbon (SOC). Therefore, the research goal was to determine how after 14 years of fertilizing, there is interaction between soil carbon sequestration and soil aggregate. Seven interventions were used in a field trial. Farmer-applied nitrogen (N), improved N executives, bio-char and manure, straw, compost, biogas waste, or enhanced N management alone is chosen. The research showed that, increasing by 30,6-120,8 % and 11,3-36,3 % in the soil layers between 0 -16 cm and 16 - 31 cm, respectively. A indicator of the soil's carbon storage process, human carbon (HUC) was one of the soil's humus carbon pools that grew by 15,7 to 206,2 % in the soil depth of 0-16 cm and between its high aromatic carbon concentration and biodegradation resistance, adding bio-char to the organic components was the most successful tactic. The concentrations of dissolved organic matter (DOC), particulate organic carbon (POC), and microbial biomass carbon (MBC) and $KMnO^{-4}$- the oxidizable carbon (ROC) all increased on average by, 122,5 %, 94,3 %, 218,2 %, and 87,5 %, respectively. The amount of exchangeable Ca^{2+},Mg^{2+}, and Fe, with soil Al oxides, the mean weight diameter (MWD),these indicators had a strong association. According to the findings, adding organic materials to calcareous soil could be a useful management technique for encouraging carbon retention and creating soil macro aggregates in the ground.

Keywords: Organic Fertilizers; Soil Aggregation; Soil Carbon Fractions; Soil Carbon Sequestration; Long-Term Fertilizing.

RESUMEN

Existe evidencia contundente de que los insumos orgánicos promueven la salud del suelo y la agricultura sostenible. Sin embargo, actualmente no está claro cómo las adiciones orgánicas de larga duración, en combinación con fertilizantes químicos, afectan la producción de macroagregados del suelo y su capacidad para almacenar carbono orgánico (COS). Por lo tanto, el objetivo de la investigación fue determinar cómo, después de 14 años de fertilización, existe interacción entre el secuestro de carbono y los agregados del suelo. Se utilizaron siete intervenciones en un ensayo de campo: nitrógeno (N) aplicado por los agricultores, ejecutivos de N mejorados, biocarbón y estiércol, paja, compost, residuos de biogás o gestión mejorada de N únicamente. La investigación mostró que, aumentando en 30,6-120,8 % y 11,3-36,3 % en las capas del suelo entre 0 -16 cm y 16 - 31 cm, respectivamente. Un indicador del proceso de almacenamiento de carbono del suelo, el carbono humano (HUC) fue uno de los depósitos de carbono del humus del suelo que creció en 15,7 a 206,2 % en la profundidad del suelo de 0 a 16 cm y entre su alta concentración de carbono aromático y resistencia a la biodegradación, agregar biocarbón a los componentes orgánicos fue la táctica más exitosa. Las concentraciones de materia orgánica disuelta (DOC), carbono orgánico particulado (POC), carbono de biomasa microbiana (MBC) y KMnO-4- el carbono oxidable (ROC) aumentaron en promedio en, 122,5 %, 94,3 %, 218,2 % y 87,5 %, respectivamente. La cantidad de Ca_{24}, Mg_{24} y Fe intercambiables con los óxidos de Al del suelo y el diámetro medio ponderal (DPM) mostraron una fuerte asociación. Según los hallazgos, la adición de materiales orgánicos al suelo calcáreo podría ser una técnica de manejo útil para fomentar la retención de carbono y la creación de macroagregados en el suelo.

Palabras clave: Fertilizantes Orgánicos; Agregación del Suelo; Fracciones de Carbono del Suelo; Secuestro de Carbono del Suelo; Fertilización a Largo Plazo.

INTRODUCTION

Compared to terrestrial vegetation, the carbon pool in the soil is 2,5 times greater, making it the greatest carbon reserve in the terrestrial biosphere.(1) SOC comprises some fractions with different turnover times, levels of breakdown, and recalcitrance. It used soil labile carbon fractions with a rapid turnover, such as ROC period, MBC, DOC, and POC, and as early indicators of how management techniques affect soil fertility is advised. Raising sulfur dioxide retention is the foundation of maintaining healthy soil. It is crucial for increasing agro ecosystem production and sustainability, as well as addressing the concerns of nutrition and environmental protection. However, in recent decades, traditional agriculture's SOC has dropped more than 30 % from pre-agricultural levels. The pyrolysis process converts sustainably produced biomass into a carbon-rich residue called bio-char.(2) it is afterward used as an agriculture amendment to boost crop yield, reduce greenhouse gas emissions, and improve SOC sequestration. Meanwhile, fertilizer can alter microbial abundance and shift SOC fractions, improving soil macro aggregate production. As a result, the soil's amount of SOC strongly correlates with soil aggregates' development and stability. However, prior research paid little consideration to the potential mechanisms by which soil aggregates can form.

Calcareous soil is the collective word for soil that includes free calcium carbonate and is found in considerable quantities in dry and semi-arid regions, which encompass approximately one-third of the physical space of the world.(3) One of the major soil types is calcareous soil, primarily composed of calcium carbonate with a negligible proportion of magnesium bicarbonate and carbonate. This specific type of soil can be recognized by its extremely low SOC concentration, large minerals in clay, and poor soil productivity.(4) Calcareous soils have an effective fixative action on phosphorus due to their elevated level of unbound carbonates of calcium and the high pH value, which leads to an extremely low level and using phosphorus in soil treatments for poor mobility. Concerning long-term soil carbon sequestration, predictions suggest that using fertilizer in combination with organic inputs can be more effective than chemical fertilizer alone.(5) To assess the SOC, humus carbon division, soil flexible carbon dioxide parts, or soil collect transport in reaction to exogenous carbon, also chosen was a field experiment to demonstrate the steps involved in creating soil macro aggregates and stabilizing the following 14 years of fertilizing on calcareous soil with various organic supplements. According to the author of (6) examine the procedure for rapid SOC, loss in limestone soils and comprehend the part that aggregate soil disturbance plays in their process.

The researchers conducted an in situ investigation in southwest China's karst region for a year. It used various tillage times, tilling the soil each 6 (T1), 4 (T2), 2 (T3), and 1 (T4) period, resulting in variable degrees of aggregate soil disruption. Implementing zero-tillage techniques and maintaining the strength of soil aggregates can reduce SOC loss, enhance soil fertility, and promote sustainable farming operations. To find out whether organic materials' uniformity was impacted by ongoing fertilization material SOC(7) associated with aggregates and how much SOC organic materials' uniformity was affected by continuous fertilization. The stabilization is caused by calcium (Ca). The effects of various fertilization methods on the stock of SOC and the special C mineralized rate (SCMR) across multiple soil fractions were compared in the research. These results imply that the creation of organo-Ca complexes can increase the availability of calcium for carbon binding during organic fertilization. It enhances soil aggregation and aids in the long-term SOC sequestration in the soils. The research Aimed to measure how adding bio-char affected different soil characteristics in calcareous soils, such as nutrients, retention of carbon, and microbial activity.[8] Sandalwood sandy and clay-based calcareous soils were treated with maize residual bio-chars generated by various temperatures for pyrolysis at two concentrations.

The following soil characteristics were assessed: pH, conductivity of electricity, nitrogen content, storage of carbon, and the fluorescein diacetate degradation activity. Plant-available potassium (K) and phosphorus (P) levels were also measured. For bio-char to function best as a natural amendment in calcareous soils, it is essential to comprehend these mechanisms. According to the author of [9] was to assess various fertilizer applications on calcareous soils with rained agriculture and straw removal. The main goal was to ascertain how would affect crop output, nitrogen agronomic effectiveness, and the soil's capacity to store both natural and total carbon. With and without adding bio-solids to plots, the research compared different amounts of mineral fertilizer ranging from 0 to 240 nitrogen fertilizing units (NFUs). The findings showed that combination fertilization, which used both mineral fertilizer and bio-solid modification, was an efficient method for lowering the use of mineral fertilizer while maintaining a similar level of crop yields.

Research looked into how managed plantation forests and naturally regenerated restoration strategies affected the sequestration of SOC in the limestone soils of the limestone. The findings of research imply that in warmer karst locations, both managed plantation forests and naturally restored shrub land vegetation can efficiently boost SOC buildup.[10] The research aimed to discover how various bio-char application rates affected the native SOC pools and microbial activities in calcareous soil over 11 years. The research conducted a field experiment in a winter wheat-maize rotation system with various biochar application rates. The research discovered that applying biochar at different rates greatly increased native SOC accumulation in calcareous soil by increasing the proportion of native resistant SOC.[11]

To find out how adding organic material and zinc fertilizer simultaneously affects the SOC and zinc uptake in wheat crops. The research tracked the amount of SOC in the soil, zinc availability in the soil and the quantity of zinc that wheat crops absorb. These results indicate the possibility of utilizing organic amendments and Zn fertilizers to enhance soil fertility and raise Zn enrichment in cereal crops in comparable agricultural settings.[12] The research examined with microbial community compositions in aggregates of calcareous soil, were affected by bio-char (BC) and chemical fertilizers. Analysis was done on the microbial community compositions, TOC and TN levels in bulk soil and various aggregate fractions, as well as aggregate stability as evaluated by MWD.[13]

According to these results, BC amendment and chemical fertilizers can work in concert to improve the soil's characteristics inside aggregates, promoting soil health and cycling of nutrients. It was utilized for stable 13C isotope studies to evaluate how adding wood ash and straw to calcareous soils affected soil carbon sequestration and the bacterial population.[14] The further research divided into four sections: part 2 outlines the methods, part 3 evaluates the efficacy of the suggested method, and part 4 summarizes the work.

METHOD

Experiment place

The research area is situated in district 1. This region experiences 24oC average annual temperature and 450 millimeters of precipitation. District's climate is classified as semi-arid, temperature, and continental monsoon. The soil's characteristics were determined to be calcium alluvial, had a pH of 9,5(w/v = 2,5:1), and had a total weight of 9,19g/kg. SOC contains 10,85 g/kg available phosphorus, 2,27 g/kg total nitrogen, and 170,00 mg/kg available potassium.

Design of Experiments

Figure 1 (a, b, c) shows the carbon, nitrogen, and pH levels of several soil amendments. Biochar has the most carbon (69,37 %) and a somewhat alkaline pH (8,33). Straw follows with 41,19 % carbon and a pH of 6,50. Manure and compost have similar carbon levels (26,08 % and 26,47 %, respectively), but differ in nitrogen concentration and pH, with manure having 1,72 % nitrogen and a pH of 7,08, while compost has 1,83 % nitrogen and a pH of 7,87. Biogas residue contains the least carbon (23,18 %) but the most nitrogen (2,12 %), with a pH of 7,23.

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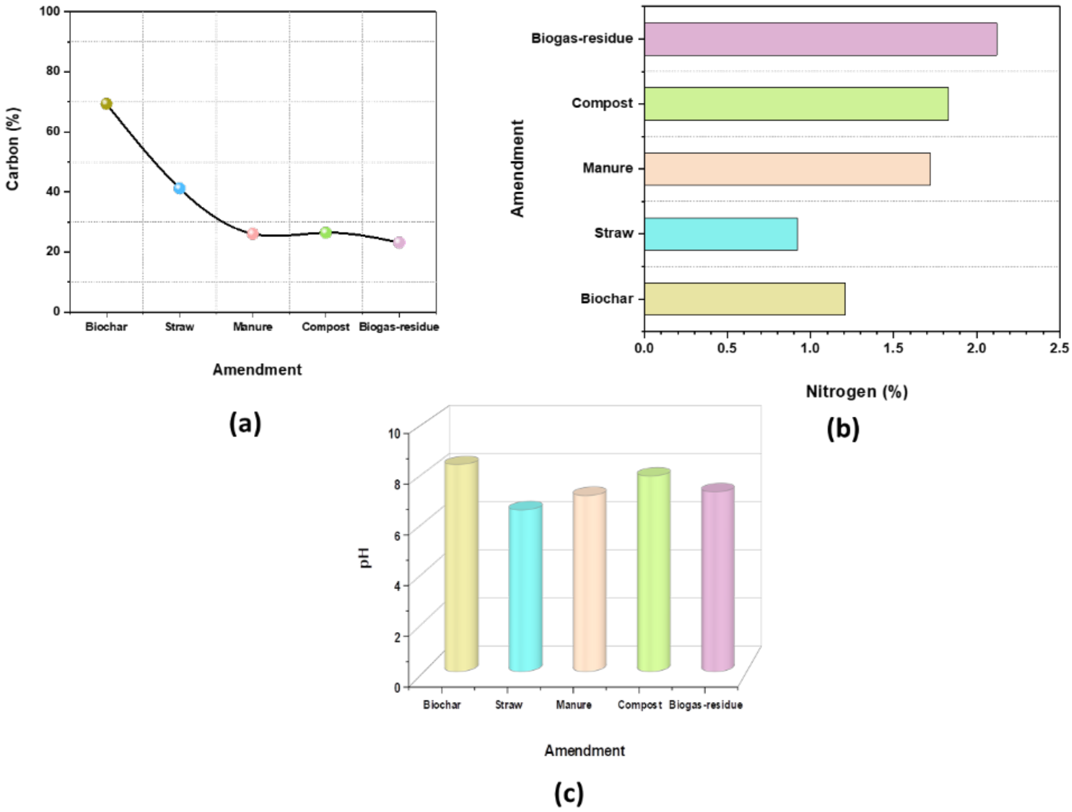

Figure 1. Different organic materials' (a) carbon (%), (b) nitrogen (%), and (c)pH values

Data collection and measurement

Following the corn harvest in 2020, soil samples were gathered at the 0-16 cm and 16-31 cm levels. Every mix involves splitting the soil samples in two, air-drying some of these fresh samples, and physically removing any visible pebbles and roots. Using a 2 or 0,25 mm screen, soil samples were separated and obtained different measures. During the MBC and DOC analysis, an additional piece of the new soil was maintained at 4°C. After being dried, ball-milled, and examined, all materials were on the sieve. Samples dried in the presence of air were incubated with 0,333 mol L^{-1} of $KMnO^{-4}$ at 25°C for 1 hour during tumbling shaking. Following centrifugation n and dilution by 1:250 with deionized water, the samples at 566 nm using a split-beam spectrometer to determine the absorption of the dilute specimens and standards Total Alkali-Extractable HE (Humid Fraction), or total alkali-extractable humid fraction, was the result. By reducing the extract's pH to 1,0 with 0,6 mol L^{-1}H2SO4 at 65°C for 1,5 hours, the HE was separated into HA(Humic Acid) and FA (Fulvic Acid), and the mixture was then left to stand at 25 °C overnight. Using the same process as for the SOC, the HUC was calculated. A TOC (Total Organic Carbon) analyzer was used to determine the carbon contents of WSSC (Water-Soluble Soil Carbon), FAC(Fulvic Acid Carbon), and HAC (Humic Acid Carbon), among other humus components. The Humic Extract Carbon (HEC) was calculated as equation (1):

$$HEC = HAC + FAC \qquad (1)$$

Soil samples were collected without disturbance to investigate the spatial distribution of soil particles following maize harvest. Four aggregate fractions were collected, oven-dried at 60°C, weighed, and computed from a 55,00 g wet-sieved air drying subsample (5 mm) with sieves measuring 2, 0,26, and 0,054 mm. The MWD was computed by equation (2):

$$MWD(mm) = \sum_{j=1}^{n} wj\ vj \qquad (2)$$

Here vj is the ratio of the mass of each aggregate's proportion to the total weight, its dimension, and wjthe aggregate size fractions' mean diameter (in mm).

Statistical Analysis

Data are presented as mean SE (standard error) (n = 4) to statistically compare all of the data, the LSD(least significant difference) technique was utilized for the treatments' variations in terms of SOC concentration, exchangeable captions like Fe and Al oxides, aggregate dispersion, humus carbon fractions, soil labile carbon division. SAS was used to do an on the correlation matrices of SOC, MWD, and other markers.

RESULTS AND DISCUSSION

Variation in SOC Content across Different Treatments and Soil Depths

Table 1 displays the SOC concentration (g/kg) at two depths of soil (0-16 cm and 16-31 cm) for seven treatments (T1-T7). SOC content ranges between 0-16 cm deep, with T3 having the greatest amount (~19,5 g kg^{-1}), followedby T6 (~15,0 g kg^{-1}) and T5 (~14,5 g kg^{-1}). At this depth, T2 has the lowest SOC content (~8,0 g kg^{-1}). In the deeper layer (16-31 cm), SOC content is generally lower but follows a similar trend, with T3 keeping the highest value (~10,5 g kg^{-1}) and T2 having the lowest (~6,0 g kg^{-1}). These variations indicate potential differences in soil carbon sequestration between treatments.

Table 1. SOC content of 0-16 cm and SOC content of 16-31 cm

Treatment	SOC Content (g kg^{-1}) at 0-16 cm	SOC Content (g kg^{-1}) at 16-31 cm
T1	~10,5	~7,0
T2	~8,0	~6,0
T3	~19,5	~10,5
T4	~13,0	~8,0
T5	~14,5	~8,5
T6	~15,0	~10,0
T7	~14,0	~9,5

Fertilization's Impact on the Soil's Percentages of labile carbon

Table 2 demonstrates how treatments (T1-T7) affected soil carbon percentages at two depths (0-16 cm and 16-31 cm). MBC, POC, DOC, and ROC values often rise with treatment, particularly in the top layer. T4 has the highest POC, although DOC and ROC are rather consistent among treatments. Overall, the findings show changes in carbon fractions, with higher accumulation in the topsoil.

Table 2. Levels of MBC, POC, DOC, and ROC at 0-16 and 16-31 cm depths of despair

Treatment	MBC (0-16 cm)	MBC (16-31 cm)	POC (0-16 cm)	POC (16-31 cm)	DOC (0-16 cm)	DOC (16-31 cm)	ROC (0-16 cm)	ROC (16-31 cm)
T1	0,03	0,05	2,1	1,5	0,06	0,05	3,0	0,6
T2	0,04	0,06	2,4	1,8	0,065	0,048	3,1	0,65
T3	0,05	0,07	2,5	2,0	0,067	0,049	3,2	0,7
T4	0,06	0,08	2,6	2,1	0,068	0,050	3,3	0,75
T5	0,06	0,08	2,4	2,0	0,066	0,048	3,2	0,72
T6	0,05	0,07	2,3	1,7	0,063	0,047	3,1	0,68
T7	0,04	0,06	2,2	1,5	0,060	0,046	3,0	0,65

Fertilization's Impact on the Fractions of Carbon in soil humus

In comparison to T1, every treatment markedly elevated the HAC, FAC, HEC by 11,1-71,8 %, 8,10-31,5 %, and 9,5–33,10 %, respectively, the 0–16 cm. Moreover, among the numerous chemical alterations, there were significant differences in HAC and HEC levels, and the greater HAC content was thought to be the cause of the higher HEC level. Figure 2 and 3 variations in the various fertility therapies' HAC, FAC, and HEC at a depth of 16 - 31 cm revealed similar tendencies at 0 - 16 cm.

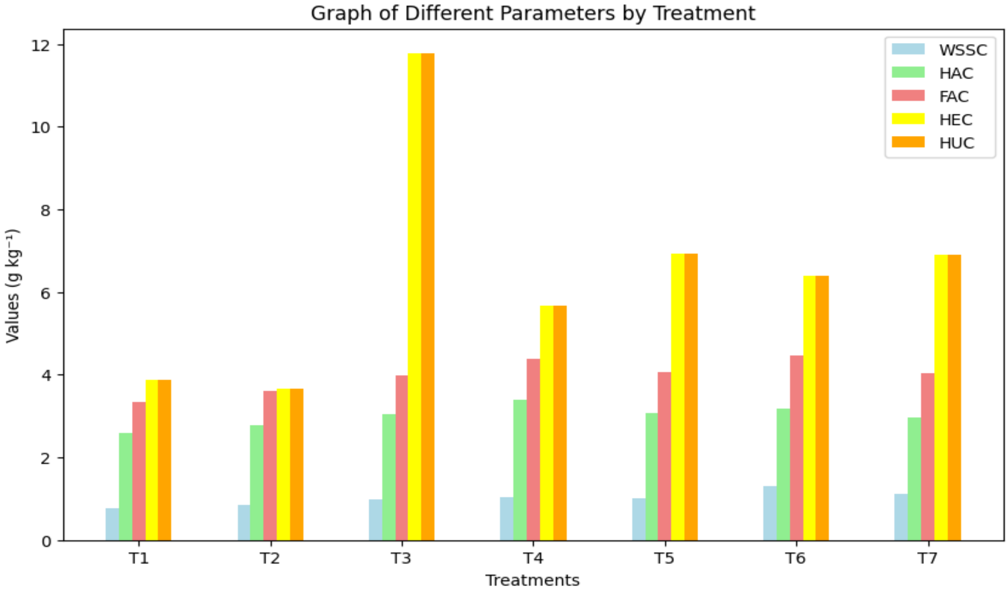

Figure 2. Effects of Different Treatments on SOC Fractions at 0-16 cm Depth

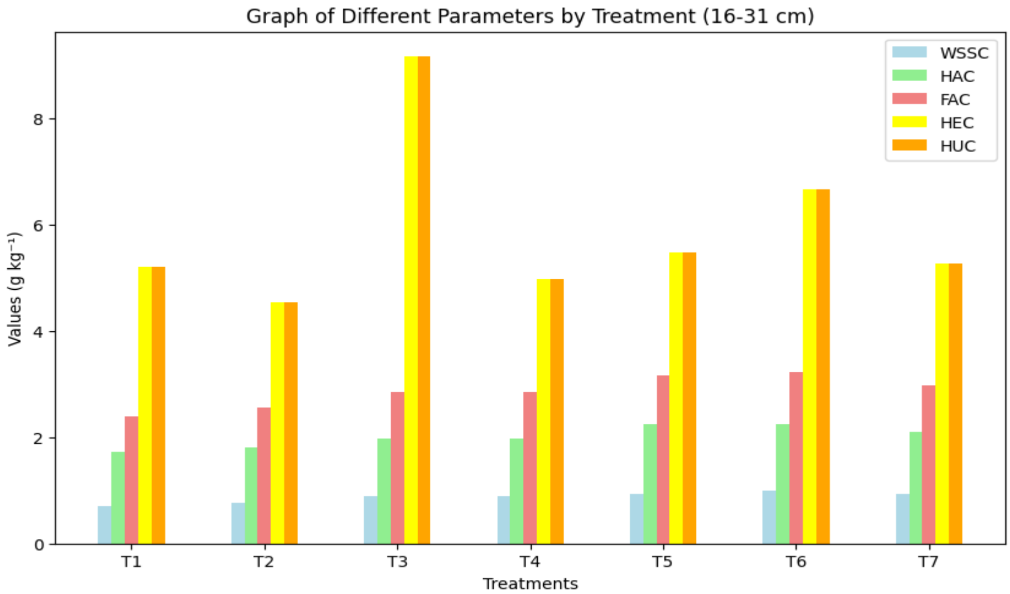

Figure 3. Effects of Different Treatments on SOC Fractions at 16-31 cm Depth

It shows the concentrations of FAC, WSSC, HAC, HUC, and total alkali- HEC at 0-16 and 16-31 cm depths in 2022 following 14 years of annual fertilization as a function of various treatments.

Fertilization's impact on the distribution of soil aggregates and means weight diameters

Table 3 shows the size distribution of soil particles in two depths ranges (0-16 cm and 16-31 cm) for distinct size categories. Fine particles (<0,053 mm) dominate both depths, however the trend is decreasing over time. The 0,25-0,053 mm fraction gradually rises, while the 2,0-2,5 mm fraction remains steady at 10 %. There are no particles larger than 2 mm at either depth. The findings show that soil composition has shifted over time, with finer particles dropping and intermediate proportions increasing.

Table 4 shows the MWD values in millimeters for two depth ranges (0-16 cm and 16-31 cm) over seven treatments (T1-T7). In Graph (a) (0-16 cm), MWD ranges from 0,20 mm (T1) to 0,37 mm (T5), suggesting heterogeneity in soil aggregate stability. MWD values in Graph (b) (16-31 cm) are generally lower, ranging from 0,15 mm (T1) to 0,22 mm (T6), indicating that deeper soil layers may have less aggregate stability than the surface layer.

The MWD in 2022 at depths of 0-16 and 16-31 cm was altered by various treatments after 14 years of annual fertilization.

Table 3. Distributions of soil aggregates at (a) 0-16 and (b) 16-31 cm depths								
Category	**Depth**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
0,053	0-16 cm	70	68	67	65	64	63	62
0,25-0,053	0-16 cm	20	22	22	25	26	27	28
2,0-2,5	0-16 cm	10	10	11	10	10	10	10
2	0-16 cm	0	0	0	0	0	0	0
0,053	16-31 cm	75	74	72	70	69	65	64
0,25-0,053	16-31 cm	15	16	18	20	21	25	26
2,0-2,5	16-31 cm	10	10	10	10	10	10	10
2	16-31 cm	0	0	0	0	0	0	0

Table 4. Depths of (a) 0-16 and (b) 16-31 cm, the MWD		
T	**MWD (mm) for (0-16 cm)**	**MWD (mm) for (16-31 cm)**
T1	0,20	0,15
T2	0,22	0,16
T3	0,30	0,18
T4	0,35	0,20
T5	0,37	0,20
T6	0,28	0,22
T7	0,31	0,19

Fertilization's impact on the soil's exchangeable Ca2+, Na+, and Mg2+, iron-,and Aluminum-oxides

Exchangeable cations like calcium (Ca^{2+}),sodium Na^{+} and magnesium Mg^{2+}in the soil can be affected by fertilization, potentially changing their concentrations and availability for plant uptake. Figure 4 and 5 dynamics of iron and aluminum oxides, which are essential for soil fertility and nutrient retention, can also be affected. changes in the soil's exchangeable calcium Ca2+, magnesium Mg2+, sodium Na+, iron oxide, and aluminum oxide content between the depths of 0 -16 cm as a result of various treatments in 2022, 14 years after annual fertilization.

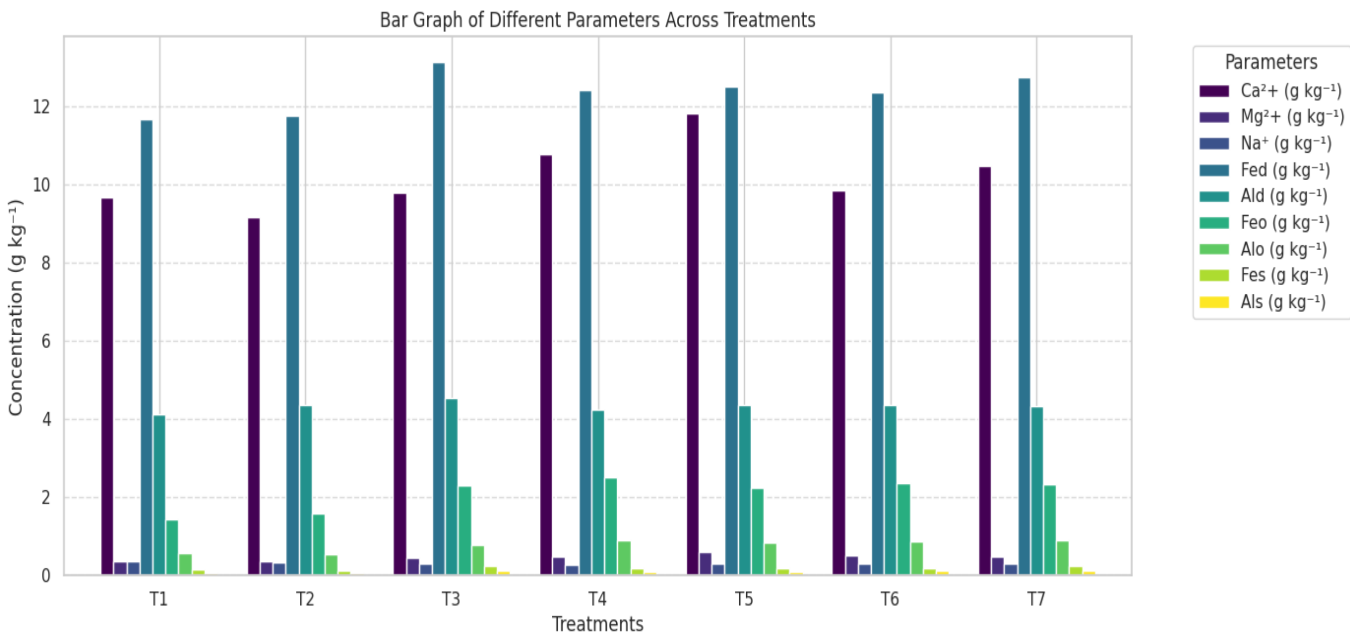

Figure 4. Soil Chemical Properties at 0-16 cm Depth Under Different Treatments

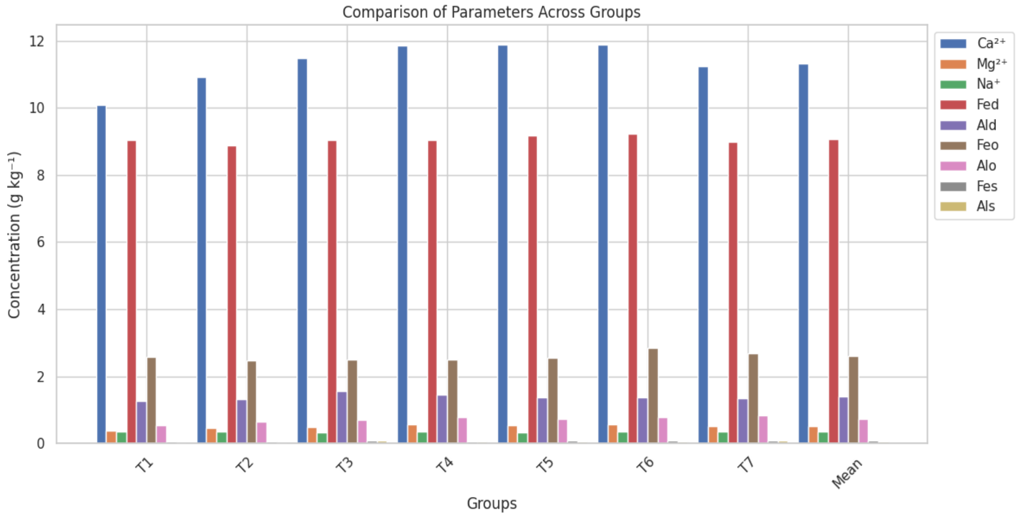

Figure 5. Soil Chemical Properties at 16-31 cm Depth Under Different Treatments

Table 5 shows the exchangeable Ca^{2+},Mg^{2+},Na^{+} concentrations (g/kg) and MWD values at two soil levels (0-16 cm and 16-31 cm). As exchangeable Ca^{2+}, Mg^{2+}, and Na^{+} levels rise, this shows that increasing exchangeable cation levels may help to improve soil aggregation in top soils, while subterranean aggregation stays relatively modest.

Table 5. Relationship Between Exchangeable Ions and MWD

Exchangeable Ca^{2+} (g/kg)	MWD (0-16 cm)	MWD (16-31 cm)	Exchangeable Mg^{2+} (g/kg)	MWD (0-16 cm)	MWD (16-31 cm)	Exchangeable Na^{+} (g/kg)	MWD (0-16 cm)	MWD (16-31 cm)
9,0	0,36	0,16	0,35	0,28	0,15	0,26	0,42	0,24
10,0	0,42	0,20	0,40	0,32	0,19	0,28	0,38	0,21
11,0	0,45	0,23	0,45	0,35	0,22	0,30	0,35	0,19
12,0	0,48	0,27	0,50	0,38	0,26	0,32	0,31	0,16
13,0	0,51	0,31	0,55	0,41	0,30	0,34	0,28	0,14

The link between iron and aluminum oxide concentration and MWD of soil aggregates was calculated at two depths (0-16 cm and 16-31 cm). Iron oxides have a positive association with MWD, with equations indicating that increased iron oxide content improves MWD. However, the strength of this relationship (R^2 value) was stronger in the upper layer (0,72) than the lower layer (0,47). Table 6 shows a positive association between aluminum oxide content and MWD, with R^2 values of 0,63 (0-16 cm) and 0,58 (16-31 cm), indicating that aluminum oxides contribute to soil aggregate stability, though slightly less than iron oxides in the surface layer.

Table 6. (a) Iron and (b) aluminum oxide composition relationships with MWD

Variables	Depth (cm)	Equation	R^2 Value
Iron oxides content (g kg^{-1}) MWD (mm)	0-16 cm	y = -0,6454 + 0,0636x	0,72
	16-31 cm	y = -0,6905 + 0,0748x	0,47
Aluminum oxides content (g kg^{-1}) MWD (mm)	0-16 cm	y = 0,6585 + 0,1829x	0,63
	16-31 cm	y = 0,1080 + 0,1377x	0,58

Matrix showing Correlation between MWD, Labile Carbon Fractions, and SOC

The correlation research revealed significant relationships between SOC, humus carbon fractions, soil aggregates, and inflexible carbon fractions at different depths. In the 0-16 cm soil layer, SOC was highly connected with humus carbon fractions and soil aggregates, indicating active carbon cycling and organic matter decomposition. Figure 6 and 7 depicts the outcomes correlation Matrix for (0-16) and (16-31).

At a depth of 16-31 cm, SOC connections with humus carbon fractions and soil aggregates were weaker, but inflexible carbon fractions showed greater interactions, indicating more persistent carbon sequestration. These variations emphasize depth-dependent variability in soil carbon dynamics, which influence long-term carbon storage and soil fertility in various soil layers.

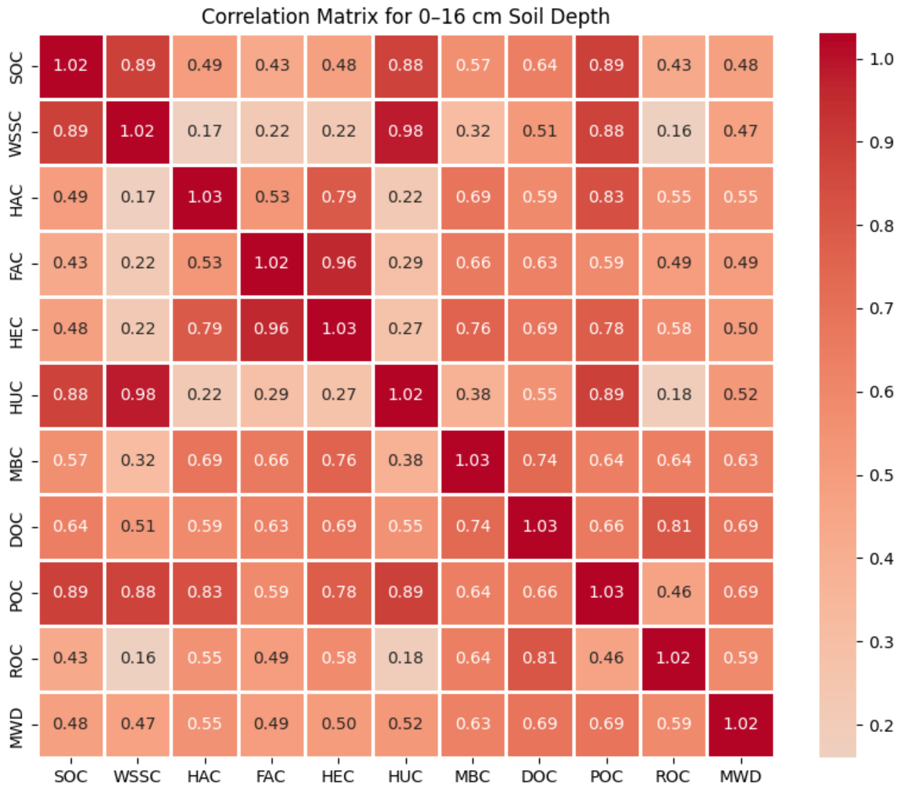

Figure 6. Correlation Matrix for 0-16 cm Soil Depth

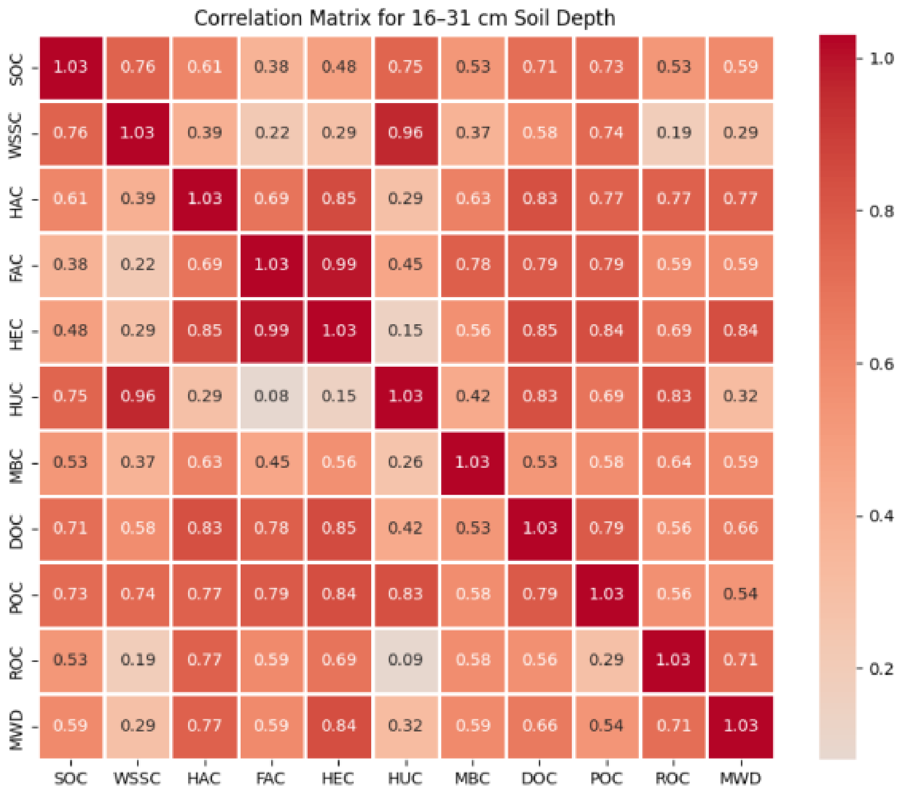

Figure 7. Correlation Matrix for 16-31 cm Soil Depth

CONCLUSIONS

Research aimed to examine how chemical fertilizers and long-term organic additions interact to affect the production of soil carbon from soil organic matter stored in macro aggregates SOC. When compared using only chemical fertilizers, adding N fertilization in addition to long-term organic amendments was found to be extremely effective for boosting sequestering, increasing soil macro aggregate production, and SOC. Due to

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their high levels of aromatic carbon, which were shown to be resistant to biodegradation, organic materials, especially bio-char, were found to be the most effective at increasing SOC concentration. The rise in soil C content was used to explain SOC content in part humus carbon pools due to adding organic materials, enhanced SOC components DOC, POC, MBC, and KMnO4,

ROC by including organic compounds. These alterations suggest increased soil fertility and the activity of bacteria. In addition, the research discovered that including organic ingredients increased the soil's exchangeable Ca^{2+},Mg^{2+},and Fe oxide levels while lowering its Na^{2+}concentration. The chemical characteristics of the earth are changing in a way that is good for the soil's health and access to nutrients. It's crucial to remember that while the research concentrated on calcareous soil, its findings might only be useful to other kinds of dirt. The unique qualities of the soil can influence the properties of the soil and how it reacts to organic amendments. The development of improved and environmentally friendly soil management techniques that support carbon sequestration, enhance soil health, and increase agricultural sustainability drive be aided by further research in this field of research.

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FINANCING

None.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

AUTHORSHIP CONTRIBUTION

Conceptualization: Priyadarshani A. Patil, Banothu Vijay, Subhaprada Dash, Harsimrat Kandhari, Geetika M. Patel, Madhur Taneja.

Data curation: Priyadarshani A. Patil, Banothu Vijay, Subhaprada Dash, Harsimrat Kandhari, Geetika M. Patel, Madhur Taneja.

Formal analysis: Priyadarshani A. Patil, Banothu Vijay, Subhaprada Dash, Harsimrat Kandhari, Geetika M. Patel, Madhur Taneja.

Drafting - original draft: Priyadarshani A. Patil, Banothu Vijay, Subhaprada Dash, Harsimrat Kandhari, Geetika M. Patel, Madhur Taneja.

Writing - proofreading and editing: Priyadarshani A. Patil, Banothu Vijay, Subhaprada Dash, Harsimrat Kandhari, Geetika M. Patel, Madhur Taneja.