

Original Research

Exploration of Soil Carbon Sequestration in Relation to C:N:P:S Stoichiometry under Dynamic Cropping Systems

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Abstract

Climate change impacts soil carbon storage by increasing temperatures, which accelerate organic matter decomposition and reduce soil carbon retention. Elevated temperatures and extreme weather events affect organic matter stability and carbon storage by disrupting C:N:P balance. Soil carbon storage is dependent on nitrogen, phosphorus, and sulfur, which help its stabilization in soil. However, there is limited information on how C:N:P:S stoichiometry affects soil carbon storage under prevailing climatic scenarios in different cropping systems. To address this gap, a two-year field experiment was conducted at the National Agricultural Research Centre in Islamabad. The study investigated maize-wheat (cereal-cereal) and fallow-wheat (fallow-cereal) cropping systems. Results showed that the highest concentrations of soil organic carbon (SOC), carbon fractions, and available nitrogen, phosphorus, and sulfur were found in the optimum humification treatment (based on 30% of humus C:N:P:S stoichiometry) for both cropping systems. This treatment resulted in an 8% increase in maize yield under the maize-wheat system and a 13-17% increase in wheat grain yield compared to the recommended dose treatment in both systems. Additionally, soil organic carbon sequestration increased by 34-36% in the maize-wheat system and 27-33% in the fallow-wheat system under the optimum humification treatment compared to sole straw incorporation. The study concludes that optimal humification practices enhance soil carbon storage by increasing microbial activity through higher organic matter inputs, thereby boosting the agricultural productivity of major cropping systems in Punjab, Pakistan.

Keywords: carbon sequestration, cropping system, stoichiometry, soil quality, humus

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Introduction

Climate change significantly impacts agriculture, threatening both the environment and food security [1]. The predominant issue driving climate change is the emission of greenhouse gases (CO_2 , CH_4 , N_2O) into the environment. The current atmospheric concentration of carbon dioxide (CO_2) is approximately 400 parts per million (ppm), which is about 31% higher than in 1900. In response to growing concerns about the climate impacts of these emissions, global efforts are focusing on encouraging carbon (C) storage in soils to offset CO_2 emissions [2]. Agriculture has substantial potential for reducing greenhouse gas emissions, with up to 90% of this potential achievable through improved carbon sequestration [3].

Carbon sequestration involves capturing atmospheric CO_2 and securely storing it in long-lasting soil pools. Currently, about 50% of agricultural soils and 24% of soils worldwide are degraded [4]. However, there is significant potential to retain atmospheric carbon in the soil for extended periods. Terrestrial soils contain around 1500 petagrams (Pg) of carbon, which is 2.5-3 times more than the carbon in the global atmosphere or terrestrial biomass [5]. Therefore, soils can significantly reduce CO_2 emissions by acting as carbon sinks [4]. Enhancing soil carbon sequestration through increased carbon inputs and improved management practices can help safeguard existing carbon stocks and maximize carbon sequestration potential [6]. Carbon sequestration is seen as a “win-win” solution for sustainable agriculture with low abatement costs [7], offering an economical and environmentally friendly strategy to improve soil and crop productivity [8].

The presence of key elements such as nitrogen (N), phosphorus (P), and sulfur (S) is essential for stable soil organic carbon and successful carbon sequestration [4]. These elements are the building blocks of life, crucial for both ecosystems and human life [9]. Consequently, the stoichiometry of these elements in plant sources, soil organic matter, and microflora significantly influences carbon and nutrient dynamics in the soil [10, 11]. The C:N, C:P, and N:P ratios in plow soil rich in organic carbon can be reliable indicators of nutrient availability during soil development [12].

The annual rotation of wheat and maize is a significant agricultural practice. A large portion of the straw produced annually is often burned or discarded, leading to negative impacts on environmental ecosystems. However, wheat and maize straw are increasingly recognized as valuable sources of organic carbon and other minerals for soil. Incorporating straw into the soil can increase soil organic carbon, provide nutrients, and decrease CO_2 release into the atmosphere [13]. However, decomposing crop straw with a high C:N ratio (50:1 to 80:1) leads to challenges when used as organic fertilizer due to its slow decomposition and nitrogen immobilization.

Farmers usually rely on straw incorporation to promote SOC accumulation and nutrient availability under field conditions.

Thus, the current study was undertaken to investigate the carbon storage in soil under different cropping systems using C:N:P:S stoichiometry of stable humus. The maintenance of C:N:P:S stoichiometry through organic and inorganic addition helps to enhance soil C storage.

Materials and Methods

Field Experiment

An evaluation of C:N:P:S stoichiometry under maize-wheat (cereal-cereal) and fallow-wheat (fallow-cereal) cropping systems was carried out over the course of a two-year field research during 2022-2023. The research was conducted at the National Agricultural Research Centre in Islamabad, which is situated at 33°43' North and 73°50' East. In addition to being a part of the Potohar highland region, the experimental area has a climate that may be classified as either subhumid or humid continental. The average rainfall during the experimental period ranged from 5 to 400 millimeters while the average temperature varied from 9-30°C (Fig. 1). The soil of the experimental location belongs to the Gujranwala soil series and is of fine-loamy, mixed, hyperthermic Udic Haplustalf.

Crop Varieties

The test crops in this study consisted of the winter crop, Zincol-2016 wheat variety, and the summer crop, CZP-132001 maize variety. These specific crop varieties were selected for their suitability to the experimental conditions.

Nutrient Sources

Two nutrient sources, organic and inorganic, were utilized in this study. The organic sources were comprised of maize straw and wheat straw at a rate of 6 t/ha while the inorganic sources consisted of mineral fertilizers. For the maize crop, the recommended NPK dosage was 100:50:50 kg/ha, while for the wheat crop, it was 120:60:50 kg/ha.

Experimental Design and Treatment Plan

A laboratory incubation study was conducted to evaluate the optimum humification level, which corresponds to a humus C:N:P:S stoichiometry or humus ratio of 30% (C:N-12; C:P-50; C:S-70). This optimum level of humification was then implemented in the field experiment. The field study consisted of four treatments including T1: control group without any amendments; T2: application of recommended nutrient doses specific

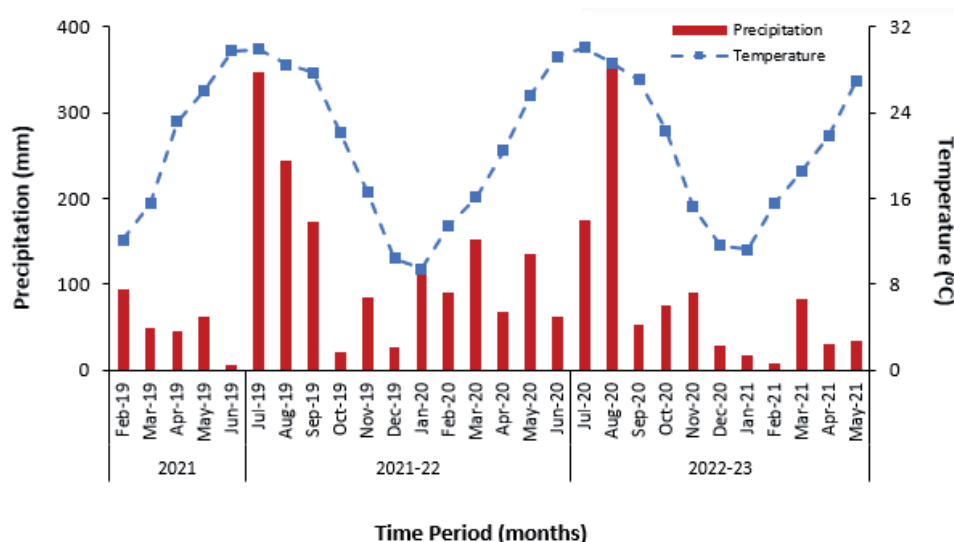


Fig. 1. Meteorological data of rainfall and temperature during experimental period (2020-2022).

to each crop; T3: Incorporation of crop straw at a rate of 6 tons per hectare (t/ha); T4: optimum humification treatment, which involved applying the 30% humification level as per the humus ratio in addition to the recommended nutrient doses. These treatments were applied to both cropping systems, namely maize-wheat and fallow-wheat. The experiment followed a split-plot design with three replications. The main plots were assigned to two cropping systems, arranged in a permanent layout. Each main plot was further divided into four subplots. The total plot size for both cropping systems was 30×21 m². Each cropping system had a main plot size of 15×15 m², which was divided into subplots of size 4×3 m², excluding the outer paths. Meteorological data, including rainfall and temperature, were collected throughout the experimental period (Fig. 1).

Data Collection

Both crops were harvested after they had reached their full maturity, and information on plant growth (plant height) and yield (grain yield and biological yield) parameters was recorded during the 2021-2022 and the 2022-2023 periods. In addition, various soil biochemical analyses were conducted as part of the field study. These included measurements of total soil organic carbon [14], carbon pools [15], microbial biomass carbon [16], available NO₃-N [17], PO₄-P [18], and SO₄-S [19] content. Soil carbon sequestration was assessed by calculating the difference between the final and initial soil organic carbon (SOC) content using the following formula:

$$\text{Soil Carbon Sequestration} = \text{Final SOC content} - \text{Initial SOC content} \quad (1)$$

Statistical Analysis

Analysis of variance (ANOVA) was applied to analyze the crop growth, yield, and fertility parameters' data using a split-plot design, and the least significant difference test was utilized to compare the means at a 5% level of significance (Steel & Torrie, 1997). With the help of the SPSS v28 program, all statistical calculations and graphing were carried out (SPSS Inc., Chicago, Illinois, 200 West Madison Street Suite 2300 Chicago, IL 60606 United States).

Results

Soil Characteristics of Experimental Site

The physical, chemical, and biological properties of the pre-sowing soil sample collected from the experimental area of NARC are given in Table 1. The soil of the experimental area belonged to the Gujranwala benchmark soil series (fine-loamy, mixed, hyperthermic Udic Haplustalf). The soil was medium in texture, alkaline (7.87) in nature, non-saline (0.53 dS/m) and it was low in organic matter content (<1%) and available nutrients (NO₃-N, PO₄-P & SO₄-S).

Soil Organic Carbon

A significant difference in TOC concentration was observed among the treatments and cropping systems (maize-wheat and fallow-wheat). However, the year effect on TOC was found non-significant. Among the cropping systems, a 2.6% increase in TOC concentration was observed in the fallow-wheat system compared to the maize-wheat system. During the first year, the maximum TOC concentration (4.49 g/kg) was found in the OH treatment, followed by RI (4.17; 4.24 g/kg)

Table 1. Physico-biochemical characteristics of the pre-sowing soil sample from the experimental site.

Soil Properties	Value
Sand	18 %
Silt	61%
Clay	21%
Texture	Silt loam
Bulk density	1.35 Mg m ⁻³
EC _e (1:1)	0.53 dS m ⁻¹
pH (1:1)	7.87
Total Organic Carbon	3.15 g kg ⁻¹
Very Labile	1.78 g kg ⁻¹
Labile	0.81 g kg ⁻¹
Less Labile	0.73 g kg ⁻¹
Non-Labile	0.86 g kg ⁻¹
MBC	141 mg kg ⁻¹
Nitrate-N	5.50 mg kg ⁻¹
Available P	3.32 mg kg ⁻¹
Sulfate-S	8.00 mg kg ⁻¹
Total N	0.04 g kg ⁻¹
Total P	0.62 g kg ⁻¹
Total S	0.53 g kg ⁻¹

and RD (3.92; 4.04 g/kg). The minimum concentration (3.66; 3.71 g/kg) was recorded in the control under the maize-wheat and fallow-wheat systems, respectively (Fig. 2). The same trend was observed during the second year under both systems.

Overall, the highest concentration of TOC (4.72 g/kg) was observed during the second year in the fallow-wheat cropping system by the treatment (OH) where crop

straw was applied in combination with supplementary nutrients to maintain humus ratio (Fig. 2).

Soil Organic Carbon Pools

The treatments and cropping systems had a significant effect on various soil organic carbon pools, such as very labile, labile, less labile, and non-labile fractions, however, the effect of the year was determined to be non-significant. In particular, in terms of cropping systems, the fallow-wheat system demonstrated greater concentrations of all carbon pools compared to the maize-wheat system over both years. Comparing the two years, the OH treatment under the fallow-wheat cropping system showed the highest concentrations of respective very labile (1.94 g kg⁻¹), labile (0.78 g kg⁻¹), less labile (0.76 g kg⁻¹), and non-labile carbon pools (0.99 g kg⁻¹) during the second year (Fig. 3a-d).

Soil Microbial Biomass Carbon

Soil microbial biomass carbon was significantly influenced by treatments, cropping systems, and years. Overall, the maximum MBC contents (271 mg kg⁻¹) were achieved in the second year through straw incorporation with supplementary nutrients in the OH treatment under the fallow-wheat system (Fig. 4). These findings suggest that the OH treatment combined with the fallow-wheat cropping system resulted in higher microbial biomass carbon, indicating improved soil health and nutrient cycling.

Soil Nutrients

The two-year average effect of cropping systems on soil nitrate nitrogen varied significantly. Fallow-wheat had 5.5% more soil nitrate nitrogen than maize-wheat (2.54 mg kg⁻¹). In general, the averaged data of two years exhibited the maximum concentration of soil nitrate nitrogen in the fallow-wheat cropping system by the OH treatment (10.95 mg kg⁻¹) during the second

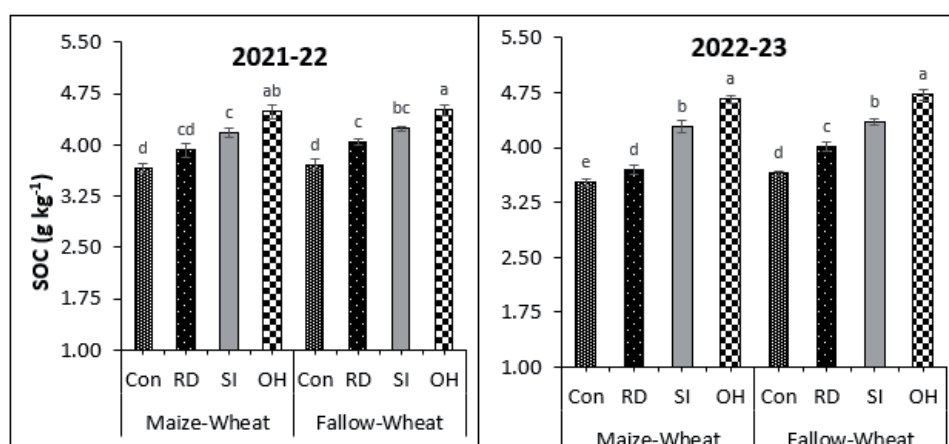


Fig. 2. Effect of treatments on SOC content under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

year (Fig. 5). Similarly, comparing two years' data, the highest concentration of available phosphorus was achieved in the second year by the straw incorporation with supplementary nutrients in OH treatment (6.53 mg kg^{-1}) under the fallow-wheat system (Fig. 6). However, the analysis of the two years' cropping

systems showed an 8.3% increase in sulfate sulfur concentration in the fallow-wheat system compared to the maize-wheat system. The fallow-wheat cropping system, particularly the optimum humification (OH) treatment, had the highest sulfate sulfur content of 10.92 mg kg^{-1} in the second year (Fig. 7).

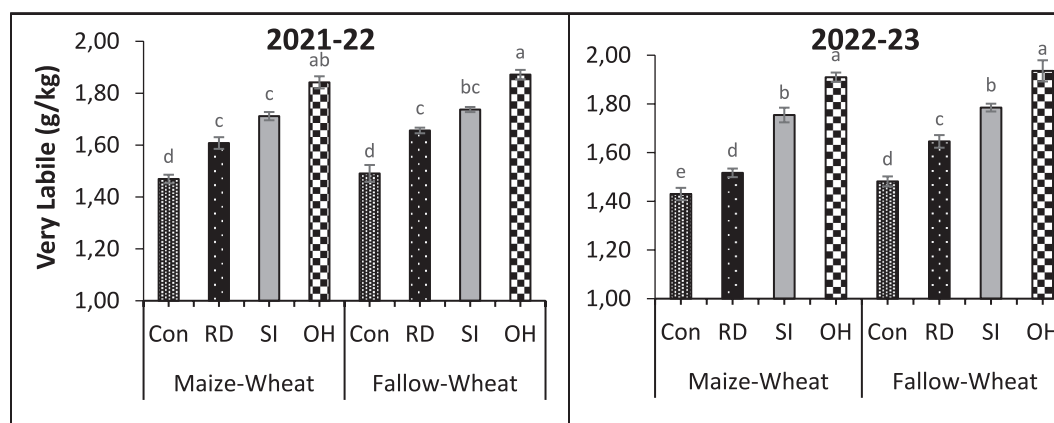


Fig. 3a. Effect of treatments on very labile SOC pool under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

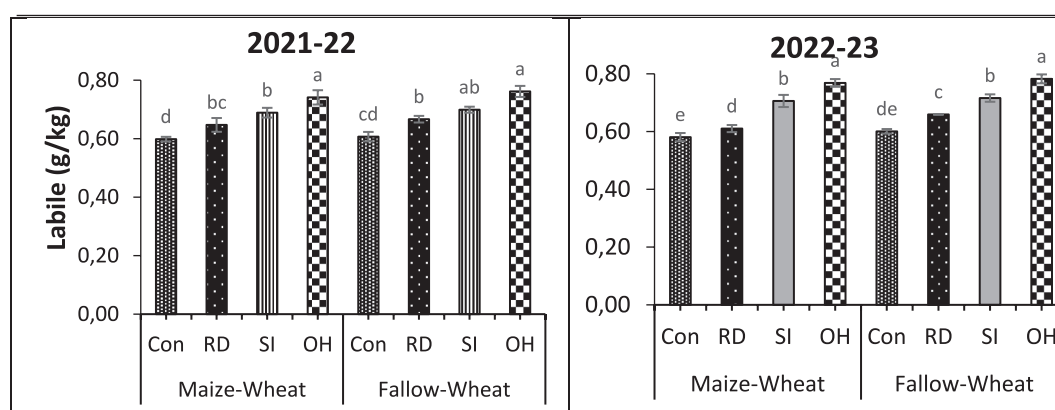


Fig. 3b. Effect of treatments on labile SOC pool under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

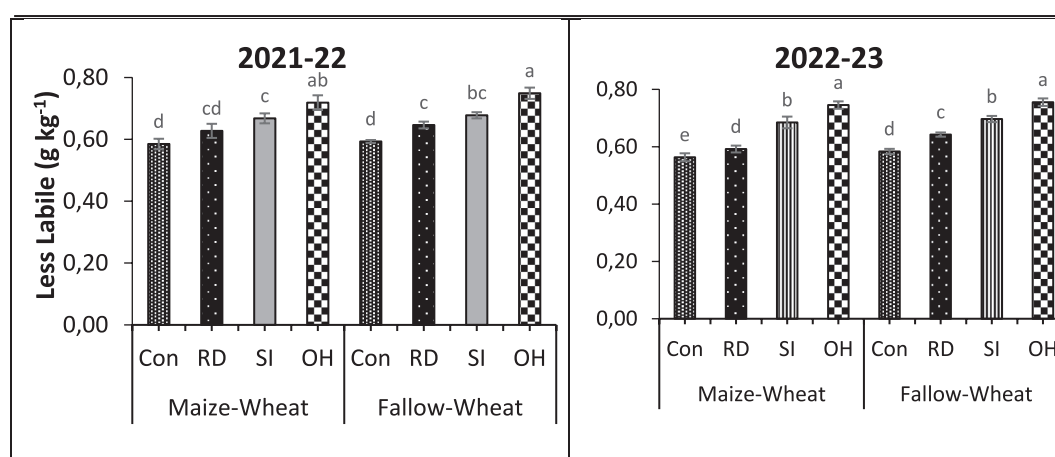


Fig. 3c. Effect of treatments on less labile SOC pool under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

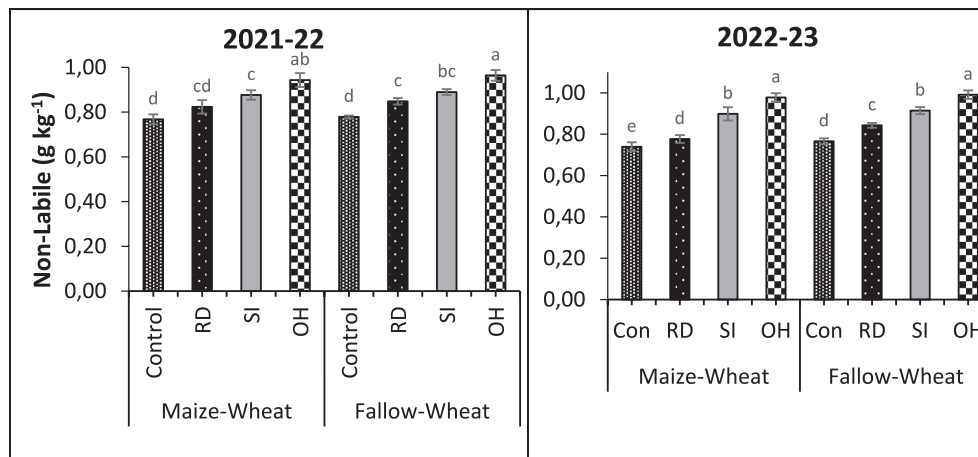


Fig. 3d. Effect of treatments on non-labile SOC pool under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

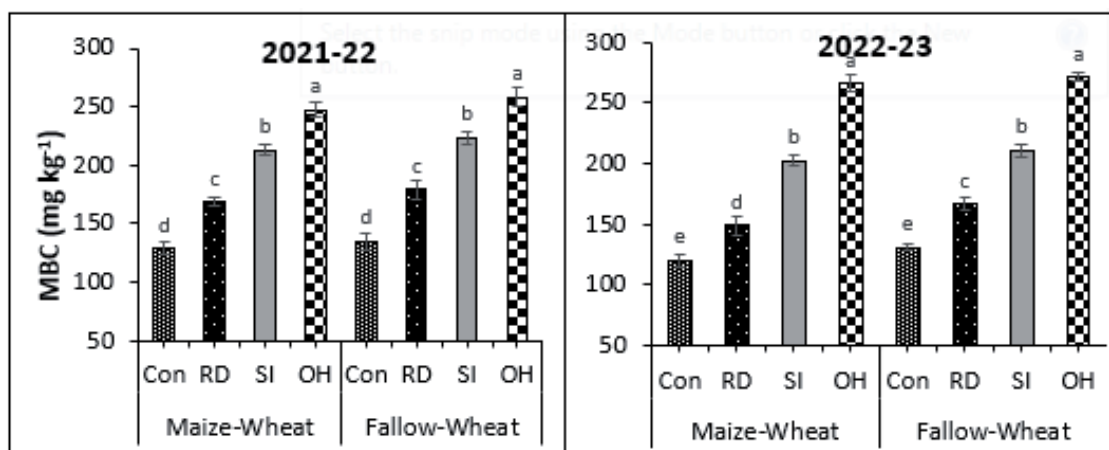


Fig. 4. Effect of treatments on microbial biomass carbon under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

Crop Growth and Yield Parameters

Plant Height

The plant height of the wheat crop was significantly ($p \leq 0.05$) influenced by the treatments, while the cropping system and year effects were found to be insignificant. Throughout both years, the plant height followed the order of $OH > RD > SI > C$ for wheat and maize crops under both cropping systems. Overall, the highest plant height for both wheat and maize crops (107 cm for wheat and 225 cm for maize) was recorded in the OH treatment under both the maize-wheat and fallow-wheat cropping systems during the second year (Fig. 8).

Grain Yield

Analysis of variance (ANOVA) showed that the grain yield of both crops, wheat and maize, was significantly ($p \leq 0.05$) affected by the treatments. However, no

significant difference was observed for cropping systems and years. In both maize-wheat and fallow-wheat cropping systems, the highest wheat grain yield (4.3; 4.4 t/ha) was obtained in the OH treatment succeeded by RD (4.2; 4.2 t/ha) and RI (3.5; 3.5cm) whereas the lowest grain yield (3.4; 3.5cm) was observed in control during the first year, respectively. A similar order of wheat grain yield increase ($OH > RD > RI > C$) was recorded under both cropping systems.

In the case of maize crop under the maize-wheat system, the maximum maize grain yield (12; 13cm) was also recorded in OH treatment following RD and RI treatments whereas the minimum (10.1; 10 cm) grain yield was in control during both years. Overall, during both years (2021–22 to 2022–23), under both maize-wheat and fallow-wheat cropping systems, the highest values of wheat and maize grain yield (107; 225 cm) were recorded in the OH treatment, where the previous crop straw was incorporated with supplementary nutrients to maintain the humus ratio (Fig. 9).

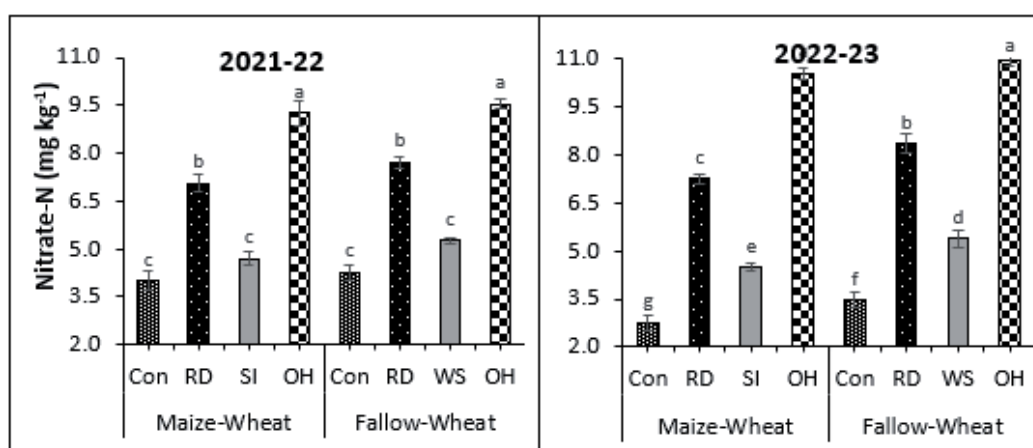


Fig. 5. Effect of treatments on soil nitrate nitrogen under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

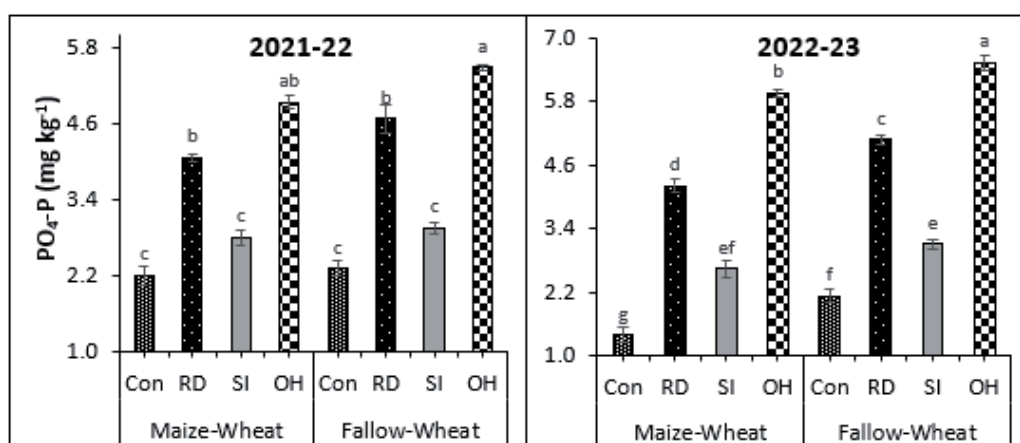


Fig. 6. Effect of treatments on soil available phosphorus under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

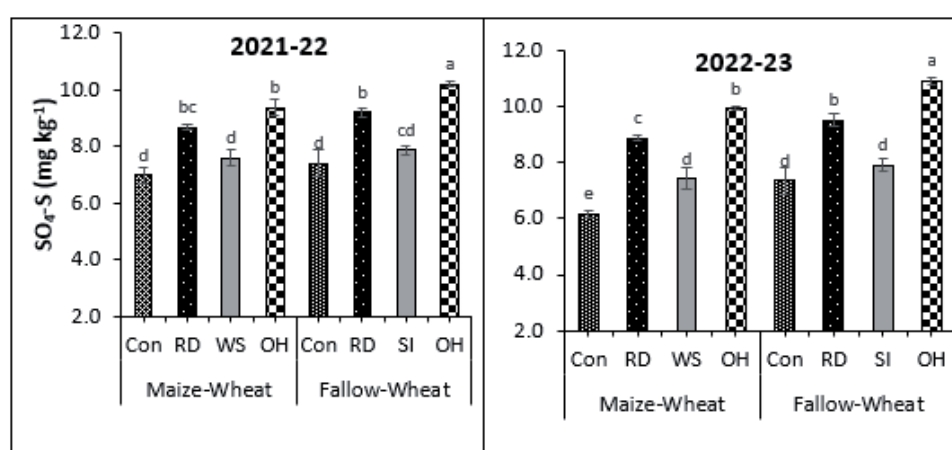


Fig. 7. Effect of treatments on soil sulfate sulfur under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

Carbon Sequestration

Across both years, the fallow-wheat cropping system demonstrated higher carbon sequestration compared to

the maize-wheat system. In the second year, the order of carbon sequestration values among treatments remained consistent under both cropping systems. Optimum humification (OH) treatment showed soil carbon

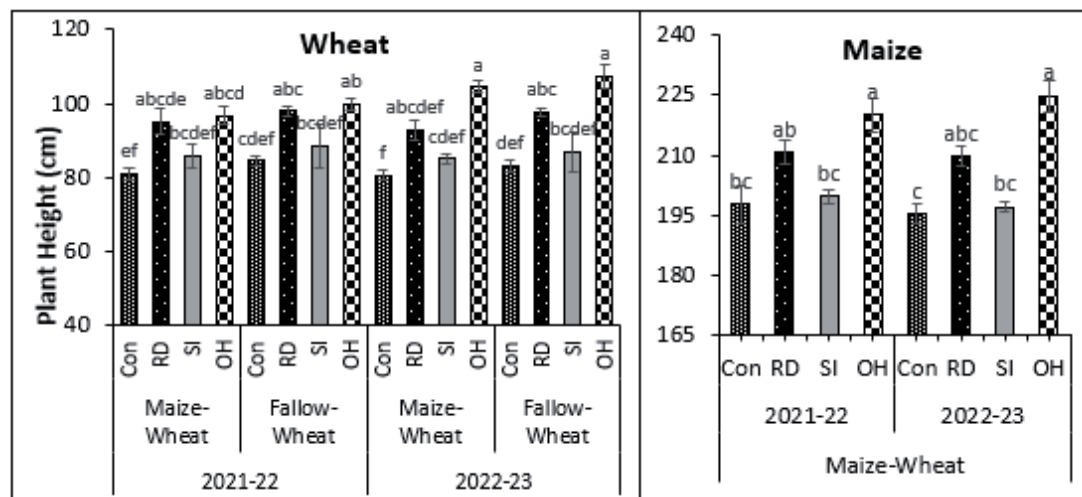


Fig. 8. Effect of treatments on wheat and maize plant height under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

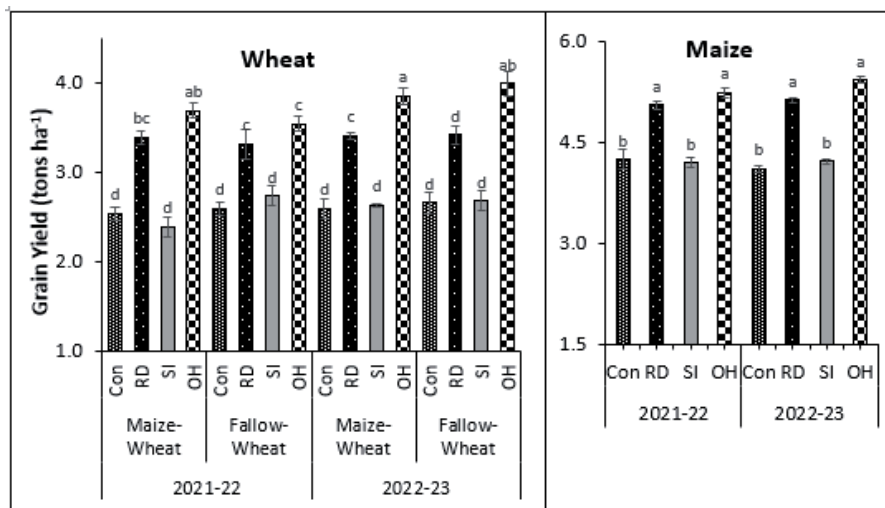


Fig. 9. Effect of treatments on wheat and maize grain yield under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

sequestration ranging from 34% to 36% in the maize-wheat and 27% to 33% in the fallow-wheat system, compared to the sole straw incorporation treatment. Comparing the data of both years, the highest carbon sequestration value of 2.98 Mg ha⁻¹ was observed in the OH treatment under the fallow-wheat cropping system during the second year (Fig. 10).

Discussion

Soil organic carbon (SOC) and carbon pools play a vital role in ensuring the long-term sustainability of agricultural systems as they not only influence soil physicochemical properties but also contribute to soil quality and health. In this study, the highest SOC contents and carbon pools, including the very labile,

labile, less labile, and non-labile pools, were observed in the treatment where crop straw was incorporated with inorganic nutrients according to the humus ratio (OH treatment). These findings align with the increase in SOC contents by the incorporation of 100% inorganic nutrients and complete crop residue [20]. The augmentation of SOC concentration through the inclusion of straw can be attributed to the accumulation of residue-derived SOC [21]. In our research, a significant increase in soil carbon pools was observed in the OH treatment compared to other treatments. The combined application of nitrogen fertilizer and straw incorporation significantly increased soil carbon pools and CO₂ emissions due to enhanced enzymatic activity [22]. Likewise, a significant enhancement of SOC and carbon fractions can be achieved with the integrated application of organic (FYM) and inorganic sources

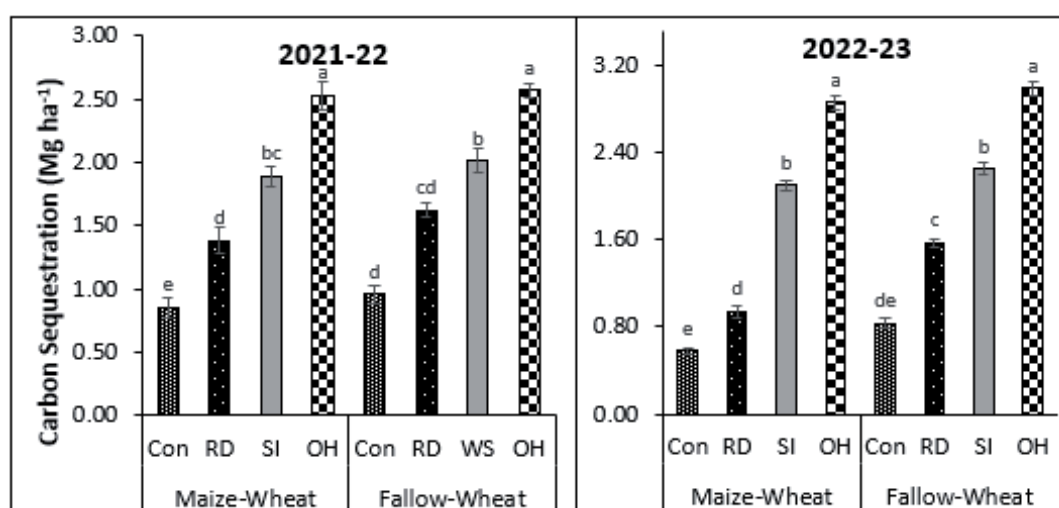


Fig. 10. Effect of treatments on soil carbon sequestration under maize-wheat and fallow-wheat cropping systems during 2021-22 and 2022-23.

i.e., NPK + FYM [23]. These SOC pools are sensitive indicators that respond quickly to short-term dynamics and can detect management-induced fluctuations [24]. Furthermore, it has been observed that the growth of root biomass and rhizodeposition also contribute to the net buildup of carbon pools in the soil [25]. These processes further emphasize the importance of SOC and carbon pools in sustaining soil fertility and ecosystem health.

Microbial biomass carbon plays a crucial role in SOM dynamics as it regulates nutrient retention and availability in the soil. It is considered a major component of the functionally active SOC pool. In our study, a high concentration of MBC was found in plots that received the OH treatment. These findings align with Sharma et al. [22], who reported that the combined use of nitrogen fertilizer and rice straw retention significantly increased the SOC, MBC, and water-extractable organic carbon. The integrated plant nutrient management treatments, particularly biochar and compost-based approaches, significantly increased MBC contents in soils [26]. Furthermore, the organic fertilizer-based balanced treatment enhanced SOC, total nitrogen, microbial biomass carbon, and nitrogen compared to the no-fertilizer treatment [27]. The strong association between MBC and SOC has been firmly established, yielding a correlation coefficient of 0.74 [28]. The influence of carbon derived from straw on MBC can be attributed to the initial soil carbon content and nutritional conditions [29]. Consequently, soil MBC serves as a highly responsive indicator for evaluating the effects of diverse soil management practices on soil quality [30]. Changes in soil management practices can have a rapid effect on carbon dynamics within the soil microbial biomass [31, 32].

Depletion of soil nutrients is a growing challenge that leads to poor soil fertility, low crop yield, and ultimately food insecurity. Extensive evidence supports the notion

that the enhancement of soil fertility and soil health can be achieved through the synergistic utilization of organic and inorganic fertilizer sources. According to the findings of our research, the addition of crop straw and chemical fertilizer to the soil at the same time, with the goal of achieving a humification level of 30% carbon in the soil, led to a substantial rise in the amount of soil nutrients, such as N, P, and S. Our findings are in line with those of an earlier study conducted by Mirzaei et al. [20], who found that the largest quantity of soil nutrients was produced by combining the crop straw with the application of 100% inorganic fertilizers. Consistent with previous research, when organic and inorganic fertilizer sources were combined, notable improvements in soil properties, such as pH, CEC, and N, P, and S contents were observed [33]. These enhancements surpassed the effects observed in treatments utilizing only mineral sources or control conditions.

Several earlier studies demonstrated that the combination of organic and inorganic sources can enhance nutrient availability through various mechanisms. The simultaneous application of organic and mineral fertilizers led to an increase in nitrogen and phosphorus concentration [34]. This increase in nitrogen concentration can be attributed to both the direct uptake of nitrogen from mineral fertilizers and the decomposition of compost in the soil, releasing additional N into the system. For P, continuous application of organic sources may reduce P fixation, increase P mineralization from compost, and contribute to the solubilization of soil inorganic phosphorus through the action of organic acids [35-37].

Our research findings regarding soil sulfur concentration align with the results presented by Admas et al. [38], who reported a significant increase in available sulfur content when compost and N & S fertilizers were applied, compared to the initial soil sulfur levels. The inclusion of sulfur-containing mineral

fertilizers and enhanced sulfate mineralization from the compost could be attributed to an increase in sulfur content in treatments involving organic and mineral fertilizers [39]. Similar conclusions were drawn where the application of organic and mineral fertilizer resulted in higher sulfur content in the surface soil compared to the unfertilized control plot [40].

Increasing SOC through the combined use of appropriate fertilizer input and organic amendments has been shown to enhance crop productivity. In our study, we observed higher plant height and grain yield (maize and wheat) in the optimum humification treatment compared to the other treatments (SI, RD, and C). These findings are consistent with the research of Sharma et al. [22], who reported significant increases in rice and wheat yield with the integrated supply of organic and inorganic sources. The decomposition of crop straw contributes to increased nitrogen (N) and phosphorus (P) availability in the soil, leading to improved rice grain output [41]. Likewise, plots treated with NPK + FYM exhibited higher biomass and yield, potentially due to deeper root systems and elevated nutrient levels in the soil. The beneficial effect of straw retention on crop grain yield has also been observed in other studies [42, 43].

Agricultural soils can be a potential carbon sink by sequestering atmospheric CO₂ through the accumulation of SOC. To enhance SOC and promote carbon sequestration in agricultural soils, combining organic and inorganic nutrient sources based on the humus ratio is a recommended approach. In our study, the OH treatment resulted in higher carbon sequestration compared to other treatments in both maize-wheat and fallow-wheat cropping systems. These findings are consistent with a field study conducted over 5 years, which reported that soil organic-C (SOC) stocks to a depth of 1.6 m increased by 5.5 t C ha⁻¹ when supplementary nutrients were applied with incorporated crop straw but decreased by 3.2 t C ha⁻¹ without nutrient addition [44]. The highest soil carbon sequestration rate was found under an integrated treatment (NPK-M) compared to NP and NPK treatments in northern China [45]. Similarly, the application of the full dose of NPK along with farmyard manure significantly increased the rate of carbon sequestration from 0.37 to 0.46 Mg C ha⁻¹ yr⁻¹ [46]. Furthermore, Kirkby et al. [47] demonstrated that increasing levels of inorganic fertilizer application improved the stabilization of crop residue-derived carbon in the soil, thereby accelerating carbon sequestration. Our results showed that irrespective of the C-input, it is essential to balance the nutrient stoichiometry of added C to better match that of resistant SOM to increase SOC sequestration. This has implications for global practices and policies aimed at increasing SOC sequestration and specifically highlights the need to consider the availability of associated nutrients in building soil carbon.

Conclusions

The optimum humification (OH) treatment significantly enhanced crop yield and soil health in both maize-wheat and fallow-wheat cropping systems. The OH treatment led to an 8% increase in maize yield and a 13-17% increase in wheat grain yield compared to the recommended dose treatment. This treatment also showed the highest concentration of soil organic carbon, carbon pools, and available nutrients such as nitrogen, phosphorus, and sulfur. The OH treatment, which achieved 30% humification of carbon based on humus C:N:P stoichiometry, proved superior in promoting soil carbon sequestration, with a range of 34-36% in the maize-wheat system and 27-33% in the fallow-wheat system. These findings underscore the benefits of OH treatment in enhancing both crop productivity and soil sustainability.

Author Contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, W.Z. and A.A.; methodology, M.N. and A.K.; software, Q.H.; validation, K.S.K., S.S., and W.Z.; formal analysis, M.N., A.K., M.Y.; investigation, S.S.I.; resources, H.A.M.; data curation, M.N., M.Y., A.K.; writing – original draft preparation, M.N., M.Y.; writing – review and editing, G.A., Q.H.; visualization, A.A.; supervision, M.A.; project administration, G.A.; funding acquisition, A.A.A.

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Conflicts of Interest

The authors declare no conflicts of interest.

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