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Organic Amendments and Reduced Tillage Accelerate Harvestable C Biomass and Soil C Sequestration in Rice–Wheat Rotation in a Semi-Arid Environment

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Abstract: Rice–wheat crop rotations have high carbon fluxes. A 2-year field study in Punjab, Pakistan quantified impacts of different nutrient management on harvestable carbon biomass, crop-derived C, soil organic C sequestration (SCS), and decomposition. Treatments included different combinations of mineral fertilizer, animal manure (20 Mg ha^{−1}), and incorporated crop residue in a split-plot design under conventional tillage (CT) and reduced tillage (RT). Combined use of mineral fertilizer and manure resulted in (1) 12.56% to 53.31% more harvestable C biomass compared to use of fertilizer and manure alone and (2) 18.27% to 60.72% more crop-derived C inputs relative to using only fertilizer or manure across both tillage practices. Combined fertilizer/manure treatments also significantly enhanced SCS relative to using fertilizer alone. Using only manure increased SCS by 63.25% compared with fertilizer alone across both tillage practices. The relationship between SCS and C inputs indicated high humification (14.50%) and decomposition rates (0.46 Mg ha^{−1} year^{−1}) under CT compared to RT at 0–15 cm soil depth. At 15–30 cm soil depth, rates of humification (10.7%) and decomposition (0.06 Mg ha^{−1} year^{−1}) were lower for CT compared to RT. Combined manure/fertilizer treatments could induce high C sequestration and harvestable C biomass with reduced decomposition in rice–wheat rotations.

Keywords: C-sequestration; harvestable C biomass; residue incorporation; rice–wheat system



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1. Introduction

Long-term sustainability in agriculture depends on soil processes which can play a crucial role in mitigating global climate change as soils have a great potential to store CO₂ in the form of soil organic matter [1]. For this purpose, agricultural practices can play a significant role in increasing soil carbon sequestration [2]. Therefore, countries around the world have adopted conservation tillage systems that can be highly profitable, productive, and support sustainable agriculture.

On the one hand, practicing conservation tillage in the form of reduced tillage can accelerate soil organic carbon build-up in soils, increasing the stability of macro-aggregates, increasing water infiltration, and thus, minimizing the loss of soil organic matter (SOM) from erosion [3]. On the other hand, the productivity of major cropping systems including rice–wheat has declined over the last few decades [4]. This has been attributed to intensive tillage operations with subsequent losses of SOM, nutrients, soil aggregates, and stability of soil particles [5]. Hence, intensive tillage [6] and the sole use of mineral nutrients [7] have reduced soil organic carbon (SOC) which has resulted in the decline of soil fertility [5].

Compared to intensive tillage, conservation tillage has great potential to induce changes in SOC in the top layers of the soils in arid and semi-arid environments [8].

One of the important issues of South Asian agriculture, including in Pakistan, is the burning and removal of residues to promote good seedbeds and to reduce chances of crop yield losses from infestation of insect pests and diseases [9]. The SOC losses through crop residue burning have been higher in rice–wheat cropping systems. This is the reason that rice–wheat cropping systems are most intensive [10] and more exhaustive [11] compared to other cropping systems. Additionally, rice–wheat cropping systems are being practiced along a vast tract of the Indo-Gangetic Plains (IGP) of South Asia totaling 13.5 million hectares (ha) including 2.2 million ha in Pakistan [12]. Recently, there has been a shift to more sustainable management practices to offset the intensity of this two-crop system.

One sustainable practice is applying farmyard manure, which not only provides valuable nutrients but also enhances the physical quality of soil for better plant nutrient and water uptake from the soil. Several published reports [4,13,14] have proposed alternative practices for re-building SOM in soils. Conservation tillage systems, animal manure application, and crop residue management [4,15,16] have been proposed as the most suitable practices to improve soil health by enhancing SOC density, infiltration capacity, and decreased soil bulk density, as well as promoting the stability of soil aggregates and SOC [8]. Crop residues and manure management play an important role in returning much-needed carbon (C) to soils, leading to higher SOC and soil quality [15]. SOC sequestration at rates of 15 to 120 kg C ha^{−1} year^{−1} were observed using nitrogen–phosphorus–potassium (NPK) fertilizer combined with manure [17]. SOC of 3.2 g kg^{−1} was reported when using farmyard manure [15]. Low SOC sequestration rates were attributed to high soil temperature, high rate of oxidation, and lower amounts of soil water.

It is well-acknowledged that agroforestry has a great potential to capture more C from the air and reduce nitrogen (N) losses from high rates of deforestation [18]. Application of organic amendments with reduced tillage practices along with agroforestry could be good strategies to increase both C and N stocks and make food systems more resilient against threats related to climate change. However, cropping systems, principally involving upland crops during one season and lowland crops during the remainder of the year, could make SOC accumulation fluctuate in soil by controlling C inputs and their decomposition rates [19]. Decomposition rates are accelerated by higher temperature but decline with increasingly hypoxic or anoxic conditions as are found in rice-based cropping systems. Parker et al., 2002 [20] observed 7–20% higher organic C in the soil of a cotton–rye system with application of poultry litter compared to that from mineral fertilizer. Furthermore, Rudrappa et al., 2006 [21] also observed a significant improvement in total SOC concentration by balanced fertilization compared to that from 50% NPK or just NP alone.

To realize significant C sequestration potential, which may be becoming more accepted in Pakistan, the adoption of best management practices is required, such as reduced or minimum tillage, integrated nutrient management, and applying animal manures. Limited reports have been published determining the effects of C inputs on harvestable C biomass and SOC sequestration rates in a dominant rice–wheat cropping rotation in Pakistan. Therefore, it is necessary to investigate the potential effects of organic amendments and tillage practices on soil organic matter as well as C and N sequestration in rice–wheat rotations under Pakistani climatic conditions. The objectives of this study were (1) to assess C biomass production, changes in soil organic matter, SOC, and N sequestration in rice–wheat rotations under tillage using different combinations of mineral fertilizer and organic amendments, and (2) to examine the quantitative relationship of C inputs with SOC sequestration rate and efficiency.

2. Materials and Methods

2.1. Site Description

Our field experiment was conducted at the research farm (31°26′24.118″ N, 73°4′29.317″ E) at an elevation of 185 m associated with the Department of Agronomy at the University of

Agriculture, Faisalabad, Pakistan. This research farm is located in the plains of central Punjab province in Pakistan. The climate of the region is semiarid subtropical continental lowland. Recent annual average precipitation is 393 mm (mm) of which 68.42% (269 mm) occurred during the summer rice growing season (June–October), while the remaining 31.58% (124 mm) took place during the winter wheat growing season (November–May). The average annual air temperature was 24.22 °C. The soil type is silt loam (19.0% sand, 54% silt, and 27% clay at a soil depth of 0–15 cm). The basic soil properties are summarized in Table 1. The dominant crops in this area are irrigated wheat, rice, sugarcane, cotton, and livestock fodder. Our experimental trial was conducted in an area where rice–wheat was already established using standard production technologies. This area was selected for soil organic carbon sequestration from the wheat season of 2014 to the end of 2016.

Table 1. Basic soil properties and initial conditions at the research farm in Faisalabad, Pakistan.

Properties	Unit	Value	
		0–15 cm	15–30 cm
OM	%	0.51	0.34
Clay	%	27	29
Silt	%	54	51
Sand	%	19	20
Stone	%	0	0
Total Nitrogen	g kg ^{−1}	0.41	0.3
Total Phosphorus	g kg ^{−1}	0.44	0.4
Total Potash	g kg ^{−1}	31.11	30.07
Available N	mg kg ^{−1}	21.32	14.7
Available P	mg kg ^{−1}	7.07	6.07
Available K	mg kg ^{−1}	164.87	138.34
Bulk Density	Mg m ^{−3}	1.49	1.51

2.2. Experimental Design and Crop Management

The cropping experiment was a split-plot design with three replicates of each treatment. Each replicate (80 × 10 m) consisted of two main-plots and sixteen sub-plots. The tillage treatments of conventional tillage and reduced tillage were randomized in main plots, and fertilization management treatments (use of mineral NPK fertilizer, crop residue, and animal manure) were allocated randomly in sub-plots. Tillage systems during the rice and wheat seasons were the conventional tillage (CT) treatment using two cultivations with the tractor pulling a cultivator along with one rotavator followed by planking for rice. For wheat, this involved three cultivations along with one rotavator followed by planking. The reduced tillage (RT) treatment consisted of one cultivation along with one rotavator for both crops. In this research study, conventional practices were those commonly used by farmers involving tilling soil to a depth of 15–20 cm from the soil surface. Farmers in Pakistan cultivate soil by using a common cultivator for the primary tillage operation. This is followed by one rotavator pass to churn the remains of the previous crop. However, one more cultivation during transplanting of rice is performed by most farmers. The secondary operation in the rice crop includes the puddling of the field in standing water to keep water stagnant during the crop growth period.

The fertilization management treatments involved using a control (T₁, Ck) with no organic or inorganic fertilizer application. Treatment 2 (T₂, NPK) used recommended doses of mineral N, P, and K. Treatment 3 (T₃) used animal manure applied at a rate of 20 Mg ha^{−1}. Treatment 4 (T₄) involved 100% incorporation of the crop residue that was left behind from previous crops. Treatment 5 (T₅, NPKM5/5) fertilization management was equally split between mineral NPK fertilizer and manure (10 Mg ha^{−1}). Treatment 6 (T₆, NPKS5/5) used 50% mineral NPK and 50% crop residue. Treatment 7 (T₇, 0.25 NPKM + 0.5 S) fertilizer application was 25% mineral NPK, 25% manure (5 Mg ha^{−1}), and 50% crop

residue. Treatment 8 (T_8 , 0.25 NPKS + 0.5 M) fertilizer application was 25% mineral NPK, 25% crop residue, and 50% M (10 Mg ha^{-1}) applied to crops.

For nutrient treatments for the T_2 application, mineral NPK application rates for rice were 140, 80, and 86 kg ha^{-1} , and for wheat these were 120, 80, and 60 kg ha^{-1} . For the T_5 and T_6 treatments, the application rate of NPK, excluding the nutrient content of straw and manure, were 60, 40, and 30 kg ha^{-1} , respectively, for wheat and were 70, 40, and 43 kg ha^{-1} for rice. For the T_7 and T_8 treatments, the NPK application rates for wheat were 30, 20, and 15 kg ha^{-1} , while for rice these were 35, 20, and 21.5 kg ha^{-1} . All P and K and one-third of N were applied at the time of seeding or transplanting. A second one-third of N was top-dressed at the tillering stage for both crops. The last one-third dose of N was top-dressed at the booting stage for both rice and wheat.

The sources of inorganic N, P, and K were urea (46-0-0), diammonium phosphate (DAP; 18-46-0), and potassium sulfate (SOP; 0-0-50), respectively. For the T_4 treatment, above-ground crop straw was collected from pre-existing rice, grown under recommended inorganic N, P, and K, after harvest. Furthermore, rice straw per unit area was estimated ($n = 3$) to range from 6000 to 7200 kg ha^{-1} . The application rate of rice straw was 6000 kg ha^{-1} , cut into 3 cm sections [7] and then returned to the field. After wheat harvest, straw yields under the T_2 treatment ($n = 3$) were estimated to range from 5500 to 6500 kg ha^{-1} . Wheat straw applied at 5500 kg ha^{-1} was incorporated into the soil before transplanting of rice. In this study, rice–wheat rotation was performed on the same field. The characteristics of organic materials are provided in Table 2.

Table 2. Basic characteristics of organic amendments applied in rice–wheat experiment at research farm in Faisalabad, Pakistan.

Source	Moisture (%)	Carbon (%)	Nitrogen (%)	Phosphorus (%)	Potash (%)	CN Ratio
Manure	39.5	29.6	0.8	0.21	0.61	37.00
Rice straw	15.5	41.31	0.98	0.18	1.62	40.31
Wheat straw	10.5	39.5	1.13	0.4	2.02	36.56

Prior to planting wheat, organic amendments were applied during the last week of November for the first (2014) and second (2015) years of the experiment. Wheat cultivar MILLAT-2011 was sown after ten days from the application of organic amendments. Wheat was harvested after mid-April during both 2015 and 2016. The rice cultivar Basmati-515 was seeded during the first week of June and transplanted before mid-July for both years. Both rice and wheat crops were harvested by cutting at 5–10 cm above the ground by a sickle.

2.3. Soil and Crop Measurements

2.3.1. Soil Analysis

Soil samples were annually collected at two depths (0–15 cm and 15–30 cm) from five points in each plot. The soil samples were air-dried, ground, and passed through a 2-mm mesh for analysis of chemical properties. The final air-dried soil samples were placed in sealed plastic jars until further analysis. Soil organic carbon (SOC) was estimated by the protocol described in Walkley and Black, 1934 [22]. Total and available N was determined by following Black et al., 1965 [23], total P by Murphy and Riley, 1962 [24], available P by Olsen et al., 1954 [25], and total as well as available K by Knudsen et al., 1982 [26]. Soil bulk density was recorded by a metal core method.

2.3.2. Determination of Harvestable Carbon Biomass and Carbon Inputs

The annual average harvestable carbon biomasses of rice and wheat were calculated by harvesting above-ground yield (Y_{grain} and Y_{straw} , Mg ha^{-1}) and measuring carbon content (C_{content} , g kg^{-1}) for straw and grain from crops, respectively [27]:

$$C_{\text{biomass}} = Y_{\text{grain}} \times C_{\text{grain}} + Y_{\text{straw}} \times C_{\text{straw}} \quad (1)$$

Total above-ground biomass and grain yield were estimated from plot yield after harvest of each crop, while straw biomass was estimated by subtracting grain yield from total biomass. Annual organic carbon input (C_{input} , Mg ha^{-1}) was determined from belowground carbon biomass ($C_{\text{belowground}}$, Mg ha^{-1}), crop stubble, plus leaf-fall ($C_{\text{s+L}}$, Mg ha^{-1}) which were incorporated into the topsoil, and C biomass from organic amendments (C_{straw} ; C_{manure} , Mg ha^{-1}) [28].

$$C_{\text{input}} = C_{\text{belowground}} + C_{\text{s+L}} + C_{\text{straw}} + C_{\text{manure}} \quad (2)$$

$$C_{\text{belowground}} = C_{\text{root}} + C_{\text{rhizodeposition}} \quad (3)$$

$$C_{\text{root}} = R_{\text{rt}} \times C_{\text{biomass}} \quad (4)$$

$$C_{\text{rhizodeposition}} = R_{\text{rd}} \times C_{\text{biomass}} \quad (5)$$

Belowground C biomass involves contribution from both crop roots and rhizo-deposition. Roots, stubble, and leaf-fall were measured after harvests of both crops, and we assumed that their proportion was steady during the whole experimental period. Here R_{rt} and R_{rd} are the ratios of C biomasses from roots and rhizo-deposits to above-ground C biomasses for each treatment. Soils with a volume of $30 \times 30 \times 30$ cm around the roots in all experimental plots were dug out and R_{rt} was determined. However, the values of R_{rd} for wheat and rice were 0.125 and 0.15, respectively, as reported by Zhao et al., 2016 [29].

$$C_{\text{s+L}} = R_{\text{s+L}} \times C_{\text{biomass}} \quad (6)$$

$$C_{\text{straw}} = B_{\text{straw}} \times C_{\text{straw}} \quad (7)$$

$$C_{\text{manure}} = B_{\text{manure}} \times C_{\text{manure}} \quad (8)$$

In Equation (6), $R_{\text{s+L}}$ is the ratio of stubble and leaf-fall C biomass to above-ground biomass. C inputs from straw and manure were estimated by multiplying the applied biomass of rice, wheat, and manure with their C-constituents.

2.4. Soil Carbon Sequestration

The soil organic carbon (SOC) difference between the initial and the final year is assumed to be the SOC sequestered (S measured in $\text{kg ha}^{-1} \text{ year}^{-1}$) under each treatment [30]. Positive and negative magnitudes of SOC were interpreted as gains and losses in SOC for all treatments, respectively:

$$S = ((\text{SOC}_t - \text{SOC}_i) \times \text{BD} \times d) / t \quad (9)$$

where SOC_t and SOC_i (g kg^{-1}) are the SOC content in the final and the initial year for treatments, respectively. The term t is the duration of the experiment (years), BD (Mg m^{-3}) is the soil bulk density, and d is the soil depth, where 0.15 and 0.30 m were used in our calculations.

2.5. Soil Nitrogen Sequestration

The soil nitrogen (SN) difference between the initial and the final year is assumed as the SN sequestered (SNS measured in $\text{kg ha}^{-1} \text{ year}^{-1}$) under each treatment. Positive and negative magnitudes of SNS were interpreted as gains and losses in SN for the treatments, respectively:

$$\text{NS} = ((\text{SN}_t - \text{SN}_i) \times \text{BD} \times d) / t \quad (10)$$

where SN_t and SN_i (g kg^{-1}) are the SN content in the final year and the initial year for the treatments, respectively. Here, t is the duration of the experiment (years), BD (Mg m^{-3}) is the soil bulk density, and d is the soil depths of 0.15 and 0.30 m used in our calculations.

2.6. Statistical Analyses

A linear regression equation ($y = b_0 + b_1x$) was applied to indicate the relationship between the SOC sequestration rate and annual C inputs under CT and RT treatments, where y versus x is the soil C sequestration rate in $\text{Mg ha}^{-1} \text{ year}^{-1}$ versus annual C input in $\text{Mg ha}^{-1} \text{ year}^{-1}$. Both b_0 and b_1 are regression coefficients of the simulation equation. A curvilinear relationship between ΔSOC (g kg^{-1}) and C inputs measured in $\text{Mg ha}^{-1} \text{ year}^{-1}$ is used to estimate apparent soil C sequestration efficiency (CSE) by applying logarithmic regression ($y = b_0 + b_1 \ln x$). Here y versus x are ΔSOC (g kg^{-1}) versus annual C input measured in $\text{Mg ha}^{-1} \text{ year}^{-1}$, while b_0 and b_1 are regression coefficients. The goodness of fit for both equations was computed by the coefficient of determination (R^2). The experiment consisted of a split-plot design, and three replications for each treatment were performed. Statistical analyses of all characteristics observed in this study were processed using two-way analysis of variance (ANOVA) following Tukey's honestly significant difference (HSD) test to compare the mean values at a probability level of 5% or less [31] in the computer-based software program Statistix 8.1 [32].

3. Results

3.1. Soil Organic Matter

At both sampling depths, SOM concentration in the rice–wheat system was the highest in organic-amended along with mineral NPK (organo-mineral treatments T_5 through T_8) plots and the least in unfertilized controls under both tillage treatments (Table 3). The SOM in plots treated with combined use of manure, straw incorporation, and mineral NPK were significantly ($p < 0.01$) higher than those from the mineral-alone NPK treatment under both reduced tillage (RT) and conventional tillage (CT) systems. For example, application of manure plus straw and mineral NPK increased SOM by 31–40% and 37–39% at the 0–15 cm and 15–30 cm soil depths compared to the control under both tillage treatments. Among treatments that just used organic amendments and inorganic NPK, the SOM concentration at both sampling depths was the most in manure-treated plots ranging from 4.60 to 6.89 g kg^{-1} . Minimum values of SOM were noted for mineral NPK treatment, which ranged from 3.82 to 5.80 g kg^{-1} . At both sampling depths, nutrient management treatments accumulated significantly more SOM under RT than CT (Table 3).

Table 3. Soil organic matter (g kg^{-1}) at two soil depths (0–15 and 15–30 cm) as affected by different fertilization treatments under CT and RT for rice–wheat.

Treatments		Soil Depth (cm)		
		0–15	15–30	Total
Conventional tillage	Control (Ck)	4.60	3.09	7.69
	Mineral NPK	5.61	3.82	9.43
	Animal manure	6.54	4.60	11.14
	Stubble/crop residue	5.92	3.99	9.92
	NPKM 5/5	7.41	5.20	12.61
	NPKS 5/5	6.40	4.61	11.01
	0.25 NPKM + 0.50 S	6.67	5.04	11.71
	0.25 NPKS + 0.50 M	7.53	4.90	12.43
Reduced tillage	Ck	5.41	3.47	8.88
	NPK	5.80	3.91	9.70
	Animal manure	6.89	4.70	11.59
	Stubble/crop residue	6.13	4.10	10.23
	NPKM 5/5	7.60	5.21	12.81
	NPKS 5/5	6.80	4.80	11.60
	0.25 NPKM + 0.50 S	6.88	5.10	11.98
	0.25 NPKS + 0.50 M	7.70	5.10	12.80

Stubble/crop residue is incorporated into the soil. NPKM 5/5 is 50% recommended NPK along with 50% manure; 0.25 NPKM + 0.5 S is 25% NPK, 25% manure, and 50% crop residue; and 0.25 NPKS + 0.5 M is 25% NPK, 25% crop residue, and 50% manure.

3.2. Harvestable C-Biomass and C-Inputs

The annual above-ground C biomass (grain plus straw) of both rice and wheat was significantly ($p < 0.01$) enhanced by fertilization treatments (Figure 1). Above-ground harvestable C biomass of rice and wheat was the highest for the NPKM5/5 treatment, ranging from 5.68 to 5.83 Mg ha^{-1} and 5.42 to 5.53 Mg ha^{-1} for rice and wheat, respectively. Above-ground harvestable C biomass was the least for the unfertilized control, ranging from 2.22 to 2.42 Mg ha^{-1} and 2.41 to 2.44 Mg ha^{-1} for rice and wheat under both tillage treatments. In terms of harvest C biomass of wheat, the NPKM5/5 treatment was statistically comparable with just NPK application and the 0.25 NPKS + 0.5 M treatment under RT (Figure 1). It was also similar to the 0.25 NPKS + 0.5 M treatment under the CT system. Use of just NPK or in combinations with organic amendments (e.g., NPK, NPKM5/5, NPKS5/5, 0.25 NPKS + 0.5 M, and 0.25 NPKM + 0.5 S) increased biomass by 50% to 62% for rice and by 44% to 56% for wheat. Compared with the control, NPK-alone or organic amendments-alone improved the above-ground C biomass production under both tillage treatments. NPK-alone and organic amendments-alone were ranked on increasing trends in above-ground C biomass: RT-NPK > RT-M > CT-NPK > CT-M > CT-S > RT-S for rice and CT-NPK > RT-NPK > CT-M > RT-M > CT-S > RT-S for wheat.

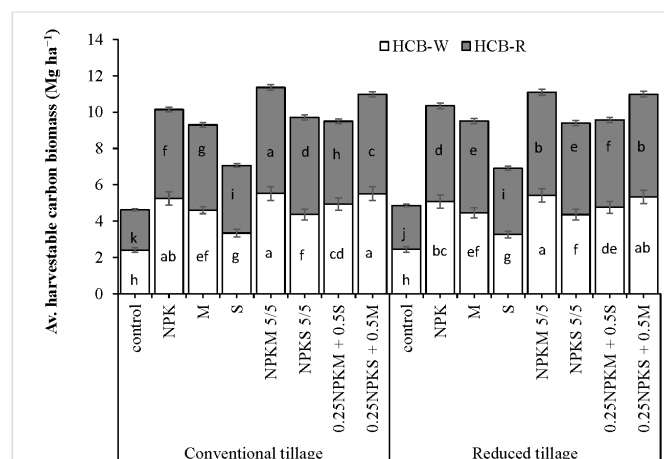


Figure 1. Average annual harvestable C biomass as influenced by different fertilization treatments under CT and RT. Each bar represents mean $\pm$ SD. Bars with unlike lower case letters (e.g., a, b, c, etc.) are statistically significant ($p < 0.05$) from each other. Solid and no-filled stacked portions show harvestable C biomass of rice and wheat, respectively. M is animal manure; S is stubble/crop residue incorporation; NPKM 5/5 is 50% recommended NPK along with 50% manure; 0.25 NPKM + 0.5 S is 25% NPK, 25% manure, and 50% crop residue; and 0.25 NPKS + 0.5 M is 25% NPK, 25% crop residue, and 50% manure.

Overall, the average annual C inputs for both rice and wheat (crop-derived C inputs) and organic amendments (externally applied) ranged from 1.31 to 10.31 $\text{Mg ha}^{-1} \text{ year}^{-1}$, equivalent to 2.61 to 20.62 Mg ha^{-1} over the experimental period (Figure 2). The average crop-derived C inputs from rice and wheat ranged from 0.74 to 2.17 $\text{Mg ha}^{-1} \text{ year}^{-1}$ and 0.57 to 1.68 $\text{Mg ha}^{-1} \text{ year}^{-1}$, respectively, equivalent to 1.48 to 4.34 Mg ha^{-1} and 1.13 to 3.39 Mg ha^{-1} over the experimental period. Similar to the above-ground C biomass, the crop-derived C inputs were also high from the NPKM5/5 treatment under both tillage systems followed by the 0.25 NPKS + 0.50 M and 0.25 NPKM + 0.50 S treatments. In the present study, organo-mineral treatments enhanced crop-derived C inputs by 54% to 66% compared with the control treatment under both tillage systems.

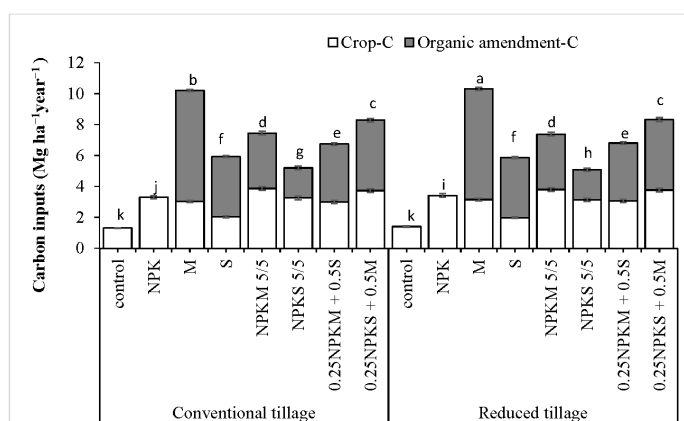


Figure 2. Average annual C inputs (roots, stubble, rhizo-deposits, straw, or manure) as affected by different fertilization treatments under conventional and reduced tillage in rice–wheat. Each bar represents mean $\pm$ SD. Bars with unlike lower case letters (e.g., a, b, c, etc.) are statistically significant ($p < 0.05$) from each other. Solid and no-filled stacked portions show C inputs from organic externally applied organic amendments and crops, respectively. M is animal manure; S is stubble/crop residue incorporation; NPKM 5/5 is 50% recommended NPK along with 50% manure; 0.25 NPKM + 0.5 S is 25% NPK, 25% manure, and 50% crop residue; and 0.25 NPKS + 0.5 M is 25% NPK, 25% crop residue, and 50% manure.

3.3. Soil Organic Carbon Sequestration

We observed increased SOC sequestration of 80.13% by organo-mineral treatments (T_5 through T_8) at a soil depth of 0–30 cm under both RT and CT systems (Figure 3a,b). Among organo-mineral treatments, the highest SOC sequestration was noted in 0.25 NPKS + 0.50 M plots followed by NPKM5/5 plots under the RT system. For example, 0.25 NPKS + 0.50 M treatment sequestered 1118.60 to 1306.01 kg C ha⁻¹ year⁻¹ at the 0–15 cm soil depth. When evaluated across tillage treatments, the C sequestration rate was 10.66% to 26.81% higher in the RT system compared to CT at both sampling depths. Application of manure enhanced SOC sequestration by 73.72% over that of the mineral NPK at the 0–30 cm soil depth. The NPK and organic amendments (T_3 and T_4) were ranked in the following order, with RT-M > CT-M > RT-S > CT-S > RT-NPK > CT-NPK.

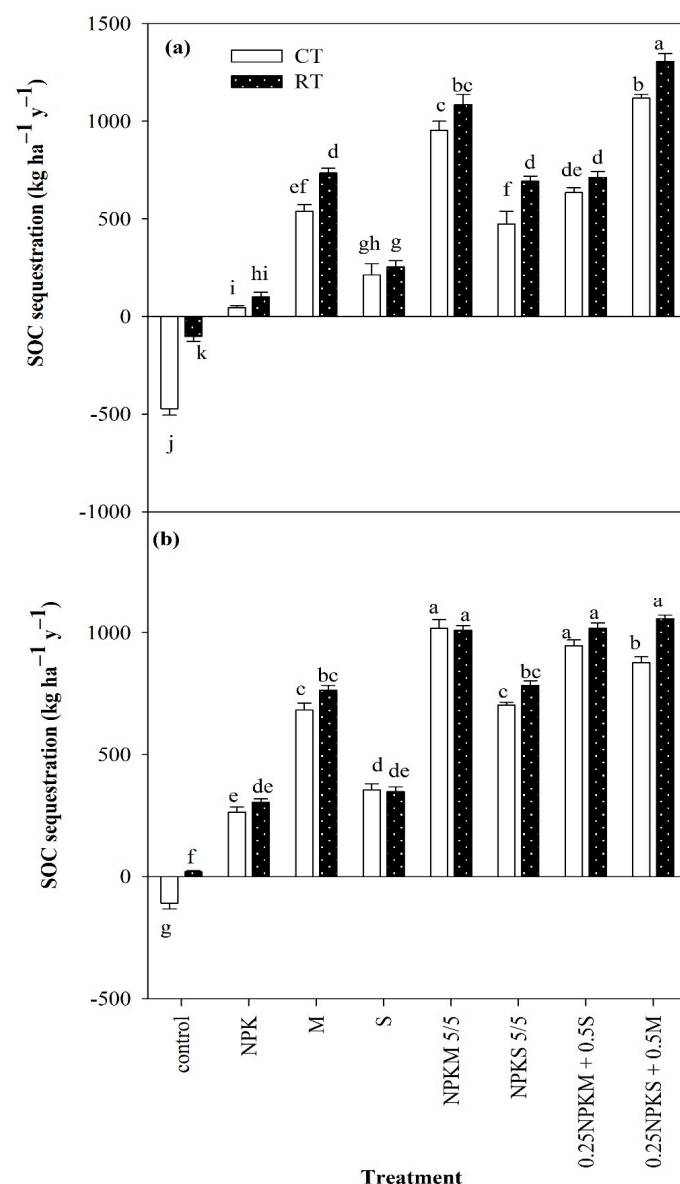


Figure 3. Average SOC sequestration rate as affected by different fertilization treatments under CT and RT at (a) 0–15 cm and (b) 15–30 cm soil depths. Each bar represents mean $\pm$ SD. M is animal manure; S is stubble/crop residue incorporation; NPKM 5/5 is 50% recommended NPK along with 50% manure; 0.25 NPKM + 0.5 S is 25% NPK, 25% manure; and 50% crop residue; and 0.25 NPKS + 0.5 M is 25% NPK, 25% crop residue, and 50% manure. Bars with unlike lower case letters (e.g., a, b, c, etc.) are statistically significant ($p < 0.05$) from each other.

3.4. Soil Nitrogen Sequestration Rate

We observed very low soil N sequestration rates from the control under both tillage treatments at a soil depth of 0–15 cm, ranging from $-82.86 \text{ kg ha}^{-1} \text{ year}^{-1}$ to $66.77 \text{ kg ha}^{-1} \text{ year}^{-1}$. The soil nitrogen accumulation rate has a maximum value of $327.99 \text{ kg ha}^{-1} \text{ year}^{-1}$ from the 0.25 NPKS + 0.5 M treatment under RT and a minimum value of $95.52 \text{ kg ha}^{-1} \text{ year}^{-1}$ for NPK-alone under CT at a soil depth of 0–15 cm (Figure 4). The organic amendments-alone enhanced N sequestration by 48.56% to 62.66% over that of the NPK-alone treatment at a soil depth of 0–15 cm. Furthermore, organo-mineral fertilization treatments enhanced N sequestration by 43.91% to 67.12% compared to NPK-alone under both RT and CT systems (Figure 4).

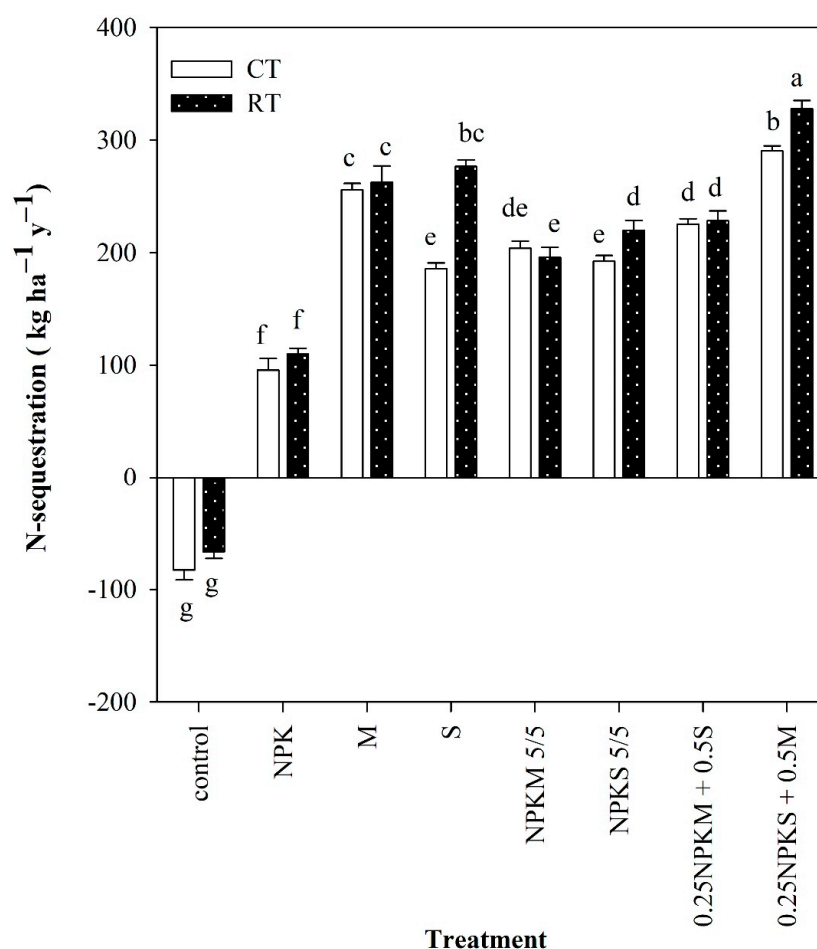


Figure 4. Influence of various nutrient management strategies on soil N sequestration rate at 0–15 cm soil depth under conventional and reduced tillage in rice–wheat cropping system. Each bar shows Mean $\pm$ SD. M is animal manure; S is stubble incorporation; NPKM 5/5 is 50% NPK along with 50% manure; NPKS 5/5 is 50% NPK along with 50% S; 0.25 NPKM + 0.5 S is 25% NPK, 25% manure, and 50% S; and 0.25 NPKS + 0.5 M is 25% NPK, 25% S, and 50% manure. Bars with unlike lower case letters (e.g., a, b, c, etc.) are statistically significant ($p < 0.05$) from each other.

At a soil depth of 15–30 cm, the N accumulation rate has a maximum value of $963.47 \text{ kg ha}^{-1} \text{ year}^{-1}$ for the 0.25 NPKS + 0.5 M treatment under RT and a minimum value of $189.91 \text{ kg ha}^{-1} \text{ year}^{-1}$ for the control under RT (Figure 5). The organic amendments-alone enhanced N sequestration by 70.75% to 79.73% relative to the control. NPK-alone also increased N accumulation by 54.97% to 67.9% compared to the control. Additionally, organo-mineral fertilization enhanced N sequestration rates by 66.59% to 80.29% relative to the control under RT and CT (Figure 5). When N sequestration was evaluated across tillage treatments, the N sequestration rate was higher in soils by 4.88% to 10.12% under RT than

CT at both sampling depths. On average, NPK-alone and organic amendments-alone were ranked in increasing order: RT-S > RT-M > CT-M > CT-S > RT-NPK > CT-NPK.

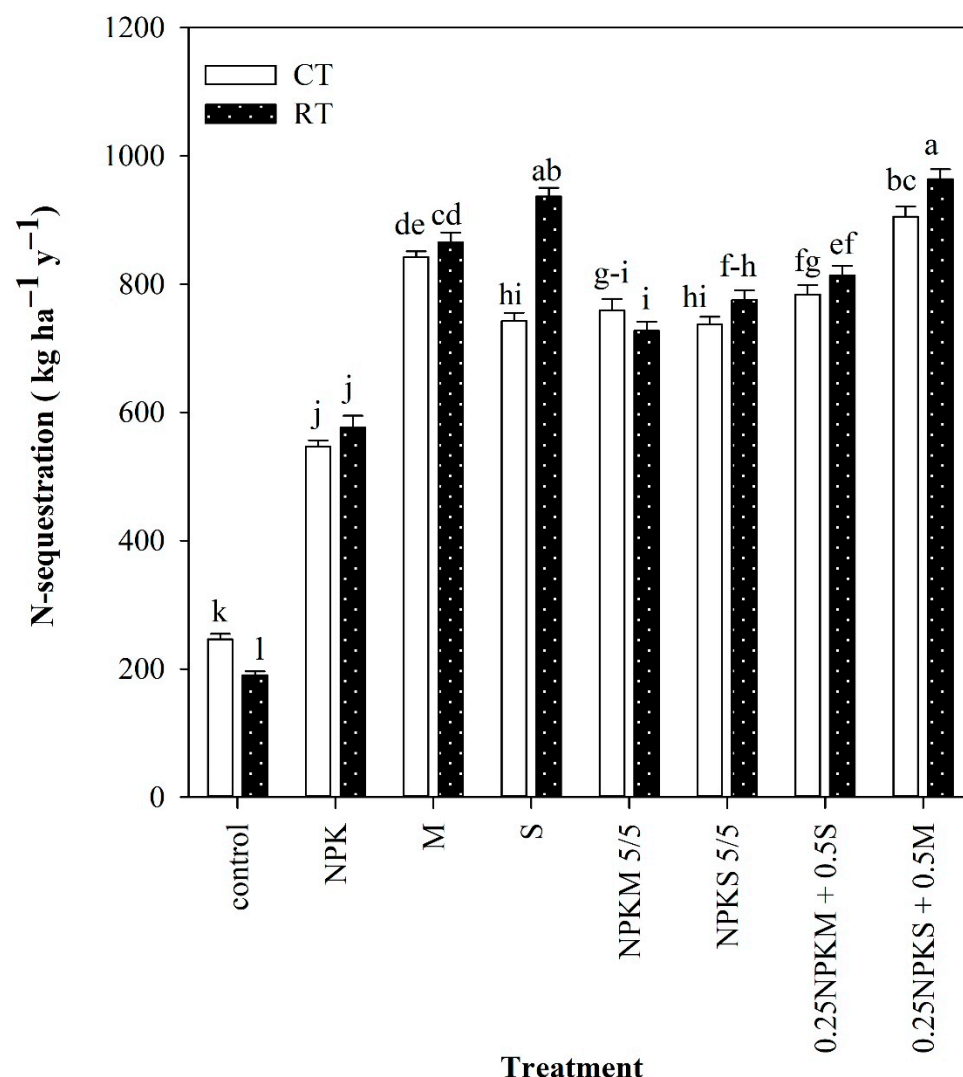


Figure 5. Effects of various nutrient management strategies on soil N sequestration rate at 15–30 cm soil depth under conventional and reduced tillage in rice–wheat cropping system. Each bar shows Mean $\pm$ SD. M is animal manure; S is stubble incorporation; NPKM 5/5 is 50% NPK along with 50% manure; NPKS 5/5 is 50% NPK along with 50% S; 0.25 NPKM + 0.5 S is 25% NPK, 25% manure, and 50% S; and 0.25 NPKS + 0.5 M is 25% NPK, 25% S, and 50% manure. Bars with unlike lower case letters (e.g., a, b, c, etc.) are statistically significant ($p < 0.05$) from each other.

3.5. Relationship between SCS and C-Inputs

A significant positive relationship between soil carbon sequestration (SCS) and C inputs was found under both tillage treatments and soil sampling depths. Conversion rate of C inputs to SOC (humification) can be computed as the slope of C sequestration against C inputs by using linear regressions (Figure 6a,b). Under the CT system, nutrient fertilization treatments resulted in high humification and decomposition rates (14.5% and 0.46 Mg ha⁻¹ year⁻¹) at 0–15 cm soil depth compared to the RT system (13.5% and 0.22 Mg ha⁻¹ year⁻¹; Figure 6a). At 15–30 cm soil depth, we observed decreased humification and decomposition rates of 10.7% and 0.06 Mg ha⁻¹ year⁻¹ for the CT system compared with that from the RT system (Figure 6b).

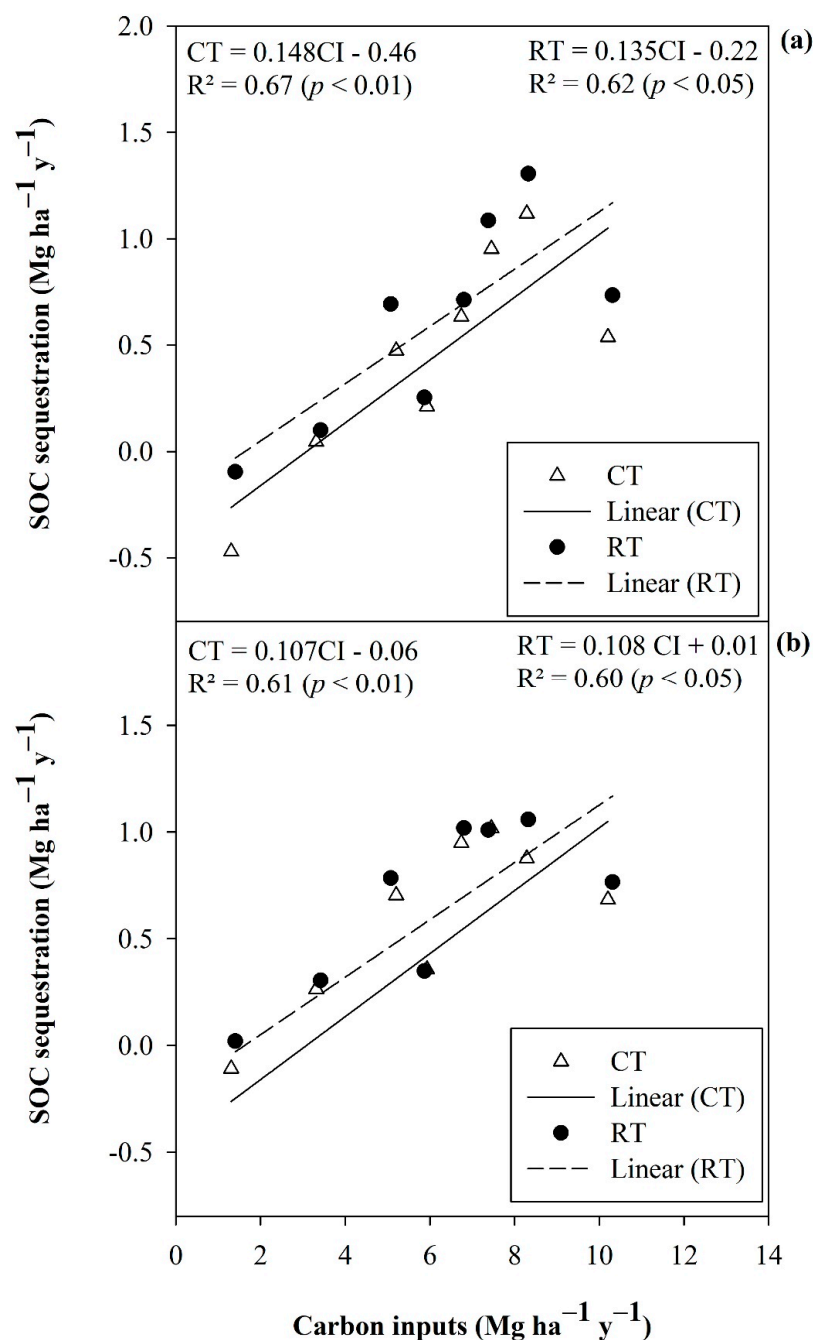


Figure 6. Relationships between SOC sequestration and annual C inputs in relation to different fertilization treatments under CT and RT treatments at (a) 0–15 cm and (b) 15–30 cm soil depths. Determination coefficients indicate significant correlation at $p < 0.05$.

3.6. Soil Carbon Sequestration Efficiency

The apparent soil carbon sequestration efficiency ($\text{CSE} = \Delta\text{SOC}/\text{C}_{\text{input}}$) during experimental periods was also affected by fertilization treatments under both tillage systems and sampling depths (Figure 7a,b). A curvilinear correlation was found between C inputs and ΔSOC contents (Figure 7a,b), representing a significant decline in marginal efficiency of C inputs. The graph (Figure 7a) indicates that 2.0 Mg ha^{-1} of C inputs were required to maintain the initial SOC level in the 0–15 cm soil layer under CT. However, less than 2.0 Mg ha^{-1} of C inputs were also needed to sustain the initial SOC level in the sub-soil layer (15–30 cm) under CT (Figure 7b). Under the RT system, reasonably fewer C inputs were needed to keep initial SOC at a constant level in both soil layers.

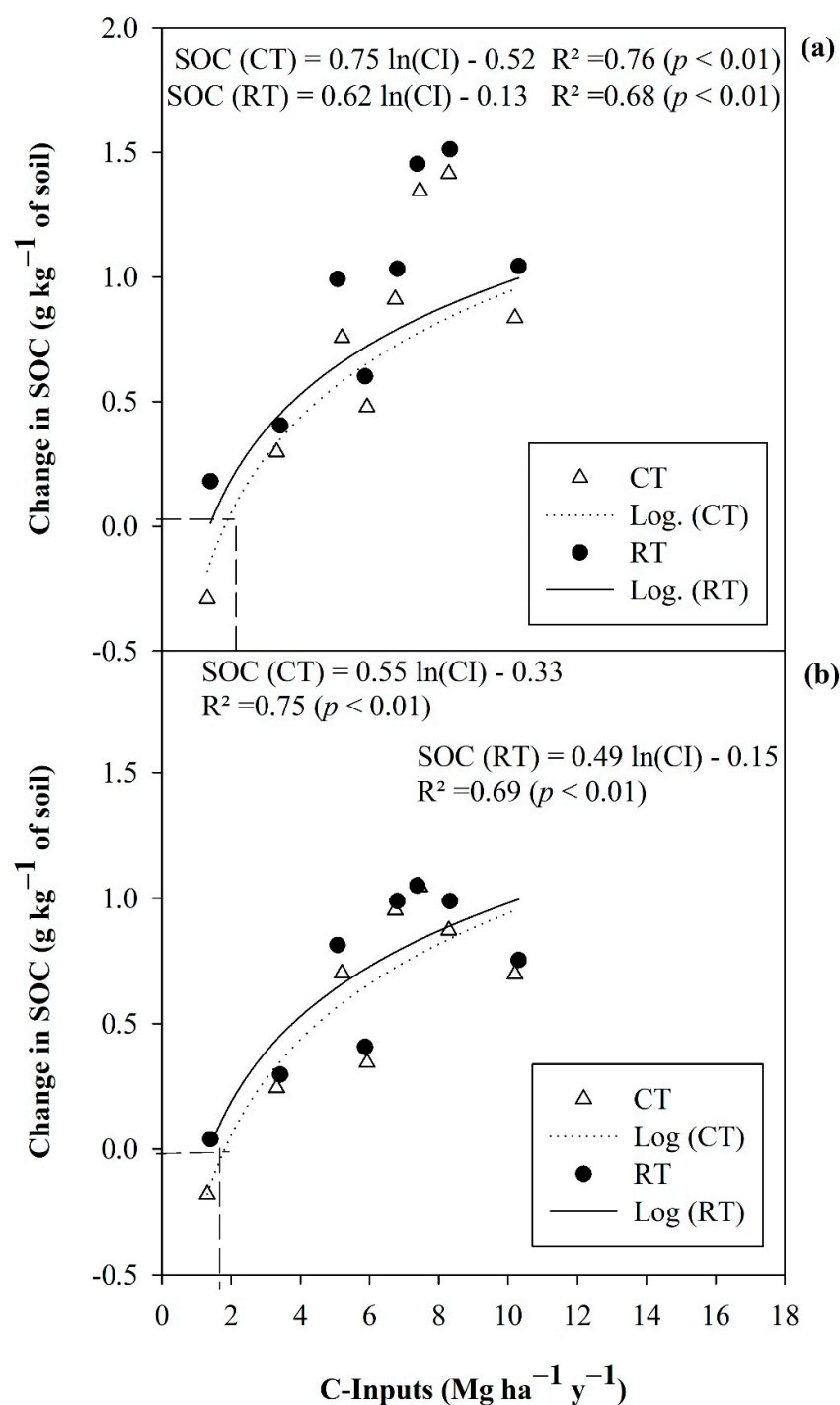


Figure 7. Relationship between soil C inputs and Δ SOC content at both soil sampling depths (a) 0–15 cm and (b) 15–30 cm as affected by different fertilization treatments under both tillage treatments. The lower dashed line indicates requirement of annual C inputs to maintain initial SOC level in the soil.

In terms of CSE, the control showed a negative CSE in both soil layers under CT, but positive CSE was also found in the control under RT. The CSE of organo-mineral treatments varied from 10.54% to 19.70% at both sampling depths. However, NPK-alone and organic amendments-alone showed a CSE ranging from 5.82% to 10.12% across both soil layers. Although C inputs from the manuring treatments were higher by 19% to 51% over those of the organo-mineral treatments, we observed maximum CSE from the NPKM5/5 treatment followed by the NPKS 5/5 treatment under both tillage systems. In the first soil layer

(0–15 cm), the trends in CSE from just using NPK and organic amendments were ranked in the following order: RT-NPK > RT-S > RT-M > CT-NPK > CT-M > CT-S.

4. Discussion

4.1. Soil Organic Matter

We observed high soil organic matter (SOM) concentrations under the reduced tillage (RT) system at 0–15 cm soil depth. Higher SOM concentrations can be accredited to less soil disturbance and decreased litter decomposition due to less soil/residue interaction [33,34]. The SOM distribution in soil can be affected by soil type, climate, and management practices [35]. The key factors influencing SOM in soils are soil temperature, soil texture, soil microbes, precipitation, tillage intensity, fertilization, irrigation, stubble management, root biomass, and soil topography [36]. Tillage methods, fertilization, and stubble management can affect soil properties such as soil porosity, infiltration rate, as well as root penetration patterns. Further tillage systems can also influence the soil physio-chemical properties in sub-soil, but their effects depend upon the tillage duration and intensity. For example, some studies revealed no significant differences in SOC concentrations in sub-soil layers under various tillage methods [33,34]. However, some researchers have indicated drastic changes in SOC in sub-soil layers [37,38]. We reported comparatively less distribution of SOM concentrations in the 15–30 cm deep soil sub-layer (Table 3).

On the other hand, conventional tillage induces more soil disturbance and enhances the SOM exposure in inter- and intra-aggregates, leading to more SOM mineralization, which suggests more incorporation and mixing of crop stubbles into the soils due to increased decomposition rates [39]. Among nutrient management systems, organo-mineral treatments improved SOM under RT at both soil sampling depths (Table 3). However, significant decreases in SOM accumulation were observed at the deeper 15–30 cm soil layer under all treatments under conventional tillage (CT). The decrease in SOC/SOM concentration with soil depths has been well documented [40]. In our current study, lower SOM in the deeper soil profile (Table 3) was likely attributed to less residue reaching the sub-soil layer.

We observed that organic matter derived from organic amendments induced more SOM accumulation in surface soil rather than in the sub-soil layer (Table 3). Meanwhile, C inputs into the subsoil layer were mostly derived from dissolved organic matter, root biomass, and root exudates [29]. Compared to the mineral NPK-alone, the incremental impact on SOM concentration was much higher using animal manure compared to straw incorporation. Furthermore, our results were corroborated by Prakash and Gupta, 2002 [41] who reported similar results due to C addition through the roots and rhizo-deposits.

The animal manure along with mineral NPK not only enriched the soils with valuable nutrients but also improved the physical environment of the soil for better uptake of both water and essential nutrients. Parker et al., 2002 [19] observed that SOM increased by 7% to 20% in the upper surface layer of soil (5 cm) under a cotton-rye system after poultry litter addition compared to commercial mineral fertilizer. In our study compared to the unfertilized control, a significant increase in SOM accumulation was recorded at both soil sampling depths by using mineral fertilizers and organic amendments together.

4.2. Harvestable Carbon-Biomass

The combined use of organic amendments and inorganic fertilizers resulted in better harvestable carbon (C) biomass of rice and wheat under both tillage systems as compared to that from manure-alone and straw incorporation (Figure 1). Mineral nitrogen deposition is necessary to enhance harvestable C biomass. Therefore, addition of nutrients either from mineral or organic sources, as well as the combination of both, increased C biomass production [42]. The nitrogen (N) addition along with organic amendments is a promising practice to enhance rapid microbial growth [27], leading to more harvestable C biomass production. However, the harvestable C biomass also varied with crop type [43]. Our results are also in accordance with those reported by Xu et al., 2011 [43].

4.3. Carbon Sequestration and Carbon Inputs

The SOC sequestration in agro-ecosystems is a complex process which is affected by several factors such as climate, soil physio-chemical conditions, fertilization strategies, and other management practices [44]. In our study, negative SOC sequestration was observed from the control under CT at both soil sampling depths (Figure 3). Because there were no external C inputs in the control, microbial breakdown of the inherent SOC exhausted the SOC stock. Thus, the final SOC stock was lower compared to the initial SOC. Furthermore, lower SOC sequestration was observed under CT, which could be attributed to the higher SOM mineralization compared to RT. Additionally, reduced tillage intensity probably leads to more aggregate stability [8], improves soil biological and biochemical processes [45], and results in higher rates of SOC sequestration.

Among organo-mineral fertilization treatments, the 0.25 NPKS + 0.50 M treatment performed substantially well under both types of tillage (Figure 3). This balanced fertilization stimulated vigorous crop growth and higher root biomass [46]. Animal manure induced positive effects on SOC content and final crop outputs [47]. We observed that use of manure enhanced SOC sequestration by 61% to 91% and 48% to 61% relative to NPK-alone and straw incorporation treatments, respectively. Animal manure stimulates crop growth and development and also enhances returns of crop residues into soils [28,48]. A linear regression relationship between SOC sequestration and C inputs were fitted at both soil sampling depths (Figure 6a,b), which suggests that SOC in our soil had not attained an equilibrium level. Therefore, soils of rice-based cropping systems in Pakistan still have a huge potential to dump more carbon. Both the linear and non-linear relationships between SOC sequestration and organic C inputs have been documented in the literature [28,49].

4.4. Carbon Sequestration Efficiency Trends

The estimation of SOC sequestration efficiency based on a logarithmic relationship of Δ SOC (sequestration) and C inputs currently under investigation (Figure 7a,b) is supported by Kong et al., 2005 [30] and Hua et al., 2014 [50]. These estimations provide us an average value for various fertilization treatments under RT and CT by assuming that soil is continuously sequestering SOC with the same efficiency irrespective of differences in management practices and C inputs. Under unfertilized control or unbalanced fertilization such as manure and straw incorporation, the limited availability of soil nutrients reduced crop growth and productivity. The SOC decomposition to supply nutrients for plant growth is inducing SOC depletion [51]. Therefore, negative soil carbon sequestration efficiency (CSE) was noted in the control under both tillage systems. However, organo-mineral fertilization induced a positive change in SOC sequestration by supplying more external C inputs in a more balanced form.

Non-linear regressions between SOC sequestration and C inputs indicated that CSE declined by supplying more than $8 \text{ Mg ha}^{-1} \text{ year}^{-1}$ of C inputs, which was observed in the manure treatment (Figure 2). On the other hand, a balanced fertilization treatment (e.g., NPKM5/5) significantly increased the harvestable C biomass (Figure 1) by supplying more soil nutrients [52], leading to more crop-derived C in the soil (Figure 2). Therefore, we observed significantly higher SOC sequestration (Figure 3a,b) with high CSE (Figure 7a,b) at both sampling depths under the NPKM5/5 treatment. Use of organo-mineral fertilization might create a suitable environment to enhance microbial growth and activity [53], resulting in rapid decomposition of additional organic matter. However, the structural composition of organic material such as manure, straw, roots, and rhizo-deposits has been a factor controlling decomposition rates [54]. In this study, contribution from belowground-derived C inputs towards the final SOC and CSE was greater compared to external C input. Some researchers indicated that root-derived C might have more chances of physico-chemical relations with soil particles, which could be retained in soils over a longer period of time [55,56].

In our current study, the range of CSE varying from −22.8% to 19.70% was comparatively lower than those ranges reported by other researchers. For example, CSE was almost

16% under a wheat–soybean cropping system in China [50] and 15.8% to 31% in mild–temperate areas under mono-culture systems [49]. Comparable results for CSE of only 6.8% to 7.7% was observed in a warm–temperate zone under a double-cropping system [49]. Rice paddy soils may sequester more SOC with a higher efficiency compared to upland soils [57]. Furthermore, drying and wetting cycles can also induce more C and N mineralization [58]. Therefore, SOC sequestration in rice-based rotations of Pakistan could be enhanced by applying more balanced nutrients to soils used for commodity crop production.

4.5. Crops, Livestock, and Agro-forestry Carbon Biomass

Expenditures for development and agricultural productivity have driven economic growth in Pakistan over the past two decades as land used for crops has increased from around 20 million hectares (ha) to between 23 to 24 million ha over this time [59]. However, agricultural land in Pakistan has become degraded due to the intensification of agricultural production as cropland shifts to pasture or crops/pasture become abandoned [60,61]. Agricultural expansion has also come at the expense of forests in Pakistan, as land use has shifted from natural habitat to more intensive land uses such as human occupation and agriculture. For example, between 1998 and 2018 in Pakistan, forests decreased from 40,937 to 36,709 ha (−10.3%), while agricultural land expanded from 4220 to 10,375 ha (+145.8%) and developed areas increased from 1498 to 5395 ha (+260.3%) [62].

Forests in Pakistan substantially differ in both their above-ground biomass as well as in their potential to store carbon. In general, carbon sequestration potential increases with Pakistani forests at higher elevation, with wider diameter at breast height, greater height, and larger crown area [63]. While dry temperate and moist temperate forests in the northern, mountainous parts of the country can have above-ground biomass in the 175 to 200 Mg ha^{−1} range, other types of forest such as (1) sub-tropical, broad-leaved and (2) dry tropical thorn can have above-ground biomass closer to 10 Mg ha^{−1} [64], which is much closer to what we measured for the combined harvestable carbon biomasses for well-fertilized rice–wheat rotations in Pakistani agricultural systems (Figure 1). This above-ground carbon storage can be combined with below-ground carbon storage in plant roots and the soil.

Future research can focus on how to better sequester carbon in agricultural systems in Pakistan, as we have demonstrated for rice–wheat at around 1 Mg ha^{−1} in soils given sufficient carbon inputs (e.g., manure) into the system (Figure 6). Considering that Pakistan has the fourth largest animal herd in the world at 196 million animals [65], there is great potential to use livestock manure for commodity crops such as rice and wheat, assuming that livestock are better integrated with these cropping systems. Even though livestock producers in Pakistan tend to be small shareholders, a recent survey of 180 small livestock farmers in central Punjab, Pakistan, found that 67.2% manage both crops and livestock to reduce risk from climate change [65]. Mahmood et al., 2019 stress the need to shift to integrated crop-livestock-forestry-fisheries production to reduce land degradation in Pakistan [66].

Improving the sustainability of rice–wheat rotations in Pakistan through better use of livestock manures and more complex integration with other agricultural enterprises such as agro-forestry can help address the needs for both woody biomass restoration and improved carbon sequestration. Agro-forestry has been shown to have more favorable soil organic matter, phosphorus, and potassium compared to re-forestation following slash-and-burn in Bangladesh [67]. Integrated crop-livestock-forest systems in Brazil have been estimated to sequester 15 Mg CO₂ ha^{−1} in soil [68]. Net carbon storage in these types of systems, such as integrated livestock–forest, can be more favorable, especially if trees are used for timber versus energy [69].

5. Conclusions

In this study, combined use of organic and mineral (organo-mineral) fertilization treatments increased harvestable carbon biomass of rice and wheat, crop-derived carbon

inputs, soil organic matter, and improved the soil organic carbon (SOC) sequestration rate and efficiency. The RT system reduced the decomposition rate, which ranged from 0.01 to 0.22 Mg ha^{−1}, and enhanced humification, which was 10.8% to 13.5%, ensuring higher SOC retention in the soils under a rice–wheat cropping system. Our regression model predicted that carbon inputs of 2 Mg ha^{−1} year^{−1} must be supplied to sustain the initial SOC level in soils occupied by rice-based cropping systems. Organo-mineral fertilization increased harvestable carbon biomass (+12.56% to +53.31%) and crop-derived carbon inputs (+18.27% to +60.72%) under both conventional and reduced tillage. Our experimental controls had negative soil organic carbon sequestration (SCS) rates (−75.28 to −579.08 kg ha^{−1} year^{−1}). Organic amendments-alone increased the SCS rate by 63.25% relative to NPK-alone under both tillage systems. Reduced tillage decreased organic material decomposition by 52.31% compared to conventional tillage. Organo-mineral fertilization can result in high carbon sequestration with reduced decomposition in rice–wheat rotations. Future research can evaluate soil carbon dynamics and sequestration potential of other cropping systems using animal manures in Pakistan and around the world, including those systems that are integrated with trees.

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