



Conservation Agriculture Practices on Carbon Stock, Carbon Sequestration and Soil Biological Properties of Rice-Okra-Green Manure Cropping System

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Conservation agriculture-based sustainable crop production intensification must not only reduce the impact of climate change on crop production but also mitigate the factors that cause climate change by reducing emissions and by contributing to carbon sequestration in soil while improving food security and enhanced productivity of resources. The present study explores the carbon stock, soil carbon sequestration potential and changes in physical, chemical and biological properties of soil due to conservation agriculture practices. Field experiments were conducted in the Agronomy Farm, College of Agriculture, Vellanikkara, from May 2019 to March 2021 to study the effect of conservation agriculture (CA) practices on carbon stock, carbon

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sequestration and soil biological properties. Rice-okra-green manure cropping system with conservation practice were compared with rice - okra cropping system without conservation practices, keeping the same layout for two years. Conservation agriculture practices had a significant influence on carbon stock, carbon sequestration and soil biological properties. After six cropping sequences, conservation practices such as direct sowing rice in flat bed + green manuring- okra+ crop residue mulch-cowpea had the maximum soil carbon sequestration of 9.52 Mg ha⁻¹ and 6.03 Mg ha⁻¹, at 15 and 30 cm soil depth, followed by direct seeding rice in flat bed + brown manuring- okra+ crop residue mulch-cowpea (10.17, 6.07 Mg ha⁻¹). The treatments with out conservation agriculture practices, such as direct seeding rice in flat beds - okra-fallow, had a negative sequestration value. The highest microbial biomass carbon (134.83 μg g⁻¹) was recorded in direct seeding rice in flat bed + brown manuring - okra + crop residue mulch-cowpea. Dehydrogenase activity was found to be significantly highest in direct seeding rice in flat bed + brown manuring - okra + crop residue mulch-cowpea (57.17 μg TPF g⁻¹ soilday⁻¹) and it was on par with direct seeding rice in flat bed + green manuring - okra + crop residue mulch-cowpea (T4) (57.17 μg TPF g⁻¹ soilday⁻¹).

Keywords: Conservation agriculture; carbon sequestration; carbon stock; soil microbial properties.

1. Introduction

Conservation Agriculture (CA) with universal principles of providing minimum soil disturbance, permanent soil cover (through crop residues/cover crops) and crop rotations is considered as a principal way to achieve goals of higher productivity while protecting natural resources and the environment. Conservation agriculture-based sustainable crop production intensification must not only reduce the impact of climate change on crop production but also mitigate the factors that cause climate change by reducing emissions and by contributing to carbon sequestration in soil while improving food security and enhanced productivity of resources. Kassam and Friedrich (2009) reported the beneficial effects of CA system as higher stable yields and income, climate change adaptation and reduced vulnerability, reduced greenhouse gas emissions, carbon sequestration, better ecosystem functioning and service with increased agro biodiversity. At present, there is no scientific data to prove the benefits of Conservation Agriculture (CA) practices for upland rice-based cropping systems. Hence, more trials and much scientific research should be done to validate and explain the merits of Conservation Agriculture (CA) practices. In this study, rice-okra-green manure cropping system with conservation practice were compared with rice - okra cropping system without conservation practices, with the specific objective to study the carbon stock, soil carbon sequestration potential and changes in physical, chemical and biological properties of soil due to conservation agriculture practices.

2. Materials and Methods

Field experiments were conducted in the Agronomy Farm, College of Agriculture, Vellanikkara, from May 2019 to March 2021 which is located at 10° 31' N latitude and 76° 13' E longitude. The soil at the experimental site was sandy loam with a pH of 4.74, an EC of 0.71 dS/m, an OC of 1.18 percent, N (174.4 kg ha⁻¹), P (63.00 kg ha⁻¹), and K (201.00 kg ha⁻¹). Rice was planted in May as the first crop, followed by okra in September as the second crop, and cowpea in January as the third crop. The experiment was carried out for two years, keeping the same layout in both years. The experiment consists of ten treatments and is replicated in randomised block design. Treatments consists of T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring-cowpea, T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch-cowpea, T3- Direct seeding rice in flat bed + Green manuring - okra + green manuring-cowpea, T4- Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch-cowpea, T5- Direct seeding rice in raised bed + Brown manuring - okra + green manuring-cowpea, T6- Direct seeding rice in raised bed + Brown manuring - okra + crop residue mulch-cowpea, T7- Direct seeding rice in raised bed + Green manuring - okra + green manuring-cowpea, T8- Direct seeding rice in raised bed + Green manuring - okra + crop residue mulch-cowpea, T9- Direct seeding rice in flat bed - okra-fallow, T10- Direct seeding rice in raised bed - okra-fallow. Paddy variety Vaisakh was used for this study. The field was ploughed by tractor in the first year. Weeds and previous crop residues were removed and levelled. Rice seeds were planted in flat bed and a raised bed. The plot size of flat bed was 5m x 4 m. For raised bed planting 3 beds (5 m x 1 m x 30 cm each) were taken in a plot area of 5 mx4m. The same layout was used for raising the subsequent crops with minimum soil disturbance. Paddy seeds were dibbled @

80 kg/ha at a spacing of 20×10 cm. The treatment T1 to T4 of rice is raised in two plots each to raise second crop okra and third crop of cowpea in T1 to T8. Cowpea seeds were also dibbled in alternate rows in treatments T1 to T4 for brown manuring and *in situ* green manuring. *In situ* green manuring was done by uprooting the cowpea plants at 25DAS and placed between the paddy rows as mulch. For brown manuring, cowpea was incorporated by spraying of 2, 4- D @ 1.25 kg ha⁻¹ at 25 DAS. Uniform hand weeding was done in all the plots at 30DAS. Soil organic carbon (SOC) data obtained from analysis of horizon-wise soil samples were used for estimating SOC stocks in pedons. The horizon wise samples from the pedons were collected as part of the profile study and the percentage organic carbon content was estimated. The bulk density data necessary for the SOC stock estimation were obtained by using the core samples collected horizon-wise. The product of bulk density (Mg m⁻³), organic carbon (%) and the depth interval gave the SOC stocks in Mg ha⁻¹ for each depth interval (Wilson & Warren, 2015).

$$\text{Carbon stock in soil (Mg ha}^{-1}\text{)} = \text{Organic carbon content} \times \text{bulk density} \times \text{depth}$$

$$\text{Carbon sequestration (Mg ha}^{-1}\text{)} = \text{Final carbon stock} - \text{initial carbon stock}$$

Soil microbial biomass carbon (µg/g soil) was extracted using the fumigation extraction method (Jenkinson and Powlson, 1976) and the Dehydrogenase activity (µg TPF g⁻¹soilday⁻¹) was estimated by Reduction of 3 % TTC to methanol-soluble formazon (TPF) and estimation using spectrophotometry (Lenhard,1953).

Data pertaining to different observations were tabulated and subjected to statistical analysis by Wasp 2.0. and the significance among the treatments was estimated at 5 per cent of probability and pooling was done for two years data.

3. Results and Discussion

3.1 Soil Carbon Stock

Soil carbon stocks was analysed after each crop at 15, 30 and 45 cm depth from all the treatments and the data presented in Tables 1,2 and 3. Results showed that the carbon stock was higher in the 15cm depth followed by 30 cm depth and the lowest in 45 cm depth in all the treatments (Figs 1,2,3). The carbon stock at 15 cm depth after all the crops was found higher in direct seeding rice in flat bed + brown manuring-okra + crop residue mulch – cowpea (T2) followed by T4- Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch-cowpea. Similar trend noticed in these treatments at 30 and 45 cm soil depth. Nideesh & Sureshkumar (2019) also reported similarly that carbon stock was comparatively less in the lower layers compared to the surface layers. After each crop the carbon stock value increased in all conservation treatments compared to control. Pursual of post-experiment data recorded the highest carbon stock at 15 and 30cm in direct seeding rice in flat bed + green manuring- okra+ crop residue mulch-cowpea (T4) of 36.72 Mg ha⁻¹ and 29.58 Mg ha⁻¹ respectively and it was on par with T2 (Table 3). At 45 cm the highest carbon stock of 18.19 Mg ha⁻¹ was observed in direct seeding rice in flat bed + brown manuring- okra+ crop residue mulch-cowpea (T2) and it was on par with T4 and T8. The treatments where the conservation agriculture practice is not followed *ie* direct seeding rice in flat bed –okra-fallow showed significantly low carbon stock after each crop at different soil depth compared to the best conservation treatments (Fig. 3). It indicated the loss of carbon in the conventional method. The carbon stock of each treatment at the end of the experiment *ie.*, after six cropping seasons (Fig. 4) indicated that there was a significant increase in carbon stock in all conservation agriculture treatments compared to the treatment without conservation practices. The highest soil carbon stock at 15 and 30 cm soil depth was recorded in direct seeding rice in flat bed + green manuring- okra+ crop residue mulch-cowpea, followed by direct seeding rice in flat bed + brown manuring- okra+ crop residue mulch-cowpea.

3.2 Carbon Sequestration

Soil carbon sequestration was analysed after each crop at 15, 30 and 45 cm depth of soil from all the treatments. Conservation agriculture practices resulted in a significant increase in organic carbon % and carbon sequestration at top soil (15 cm) compared to non conservation practice. Similar results of the beneficial effect of conservation agriculture in carbon sequestration was also reported by Kassam & Friedrich (2009). The

highest carbon sequestration was recorded for direct seeding rice in flat bed + brown manuring- okra+ crop residue mulch-cowpea. The carbon loss was recorded in the control and that might be due to the absence of conservation practices. Surface mulching by crop residues in the conservation agriculture treatments might have resulted in carbon sequestration. These results agree with the findings of Sudha & George (2011) and Thulasi et al., 2013 reported that mulching at top layers improves carbon sequestration.

From the post experiment data the highest carbon sequestration at 15 and 30 cm was observed in direct seeding rice in flat bed + green manuring- okra+ crop residue mulch-cowpea (T4) of 10.17 Mg ha⁻¹ and 6.07 Mg ha⁻¹ respectively and it was on par with direct seeding rice in flat bed + brown manuring- okra+ crop residue mulch-cowpea (T2) of 9.52 Mg ha⁻¹ and 6.03 Mg ha⁻¹ respectively (Table 6). The lowest carbon sequestration of -9.64 Mg ha⁻¹, -9.78 Mg ha⁻¹ and -4.40 Mg ha⁻¹ at 15, 30, 45 cm respectively was recorded in direct seeding rice in flat bed + okra –fallow (T9) and it was on par with direct seeding rice in raised bed-okra –fallow (T10). From the post experiment data, it was clearly evident that carbon was sequestered in all the conservation treatments both in flat bed and raised bed at 15 cm depth (Fig. 1). The treatments where the conservation agriculture practice is not followed *ie* direct seeding rice in flat bed –okra-fallow showed a negative value for sequestration than the best conservation treatments (Fig. 3, Fig. 6). It indicated the loss of carbon in the conventional method.

In 30 and 45 cm depth of the entire soil profile the sequestration for all the conservation treatments not followed the similar trend. A slow increase in sequestration after each crop was clearly observed. It indicated that conservation agriculture practices for a long run will sequester the carbon to entire soil profile (Fig. 2). This result is in consonance with the findings of Powlson et al., 2014 that a different trend was observed in depth wise distribution for a short period of conservation agriculture (less than five years) the no till increases soil organic carbon concentration compared to plough till method only on the surface layer and not for entire soil profile. For better improvement to the entire profile a long-term continuous conservation practice is required. Alvarez (2005) also reported similarly that accumulation of soil organic carbon under reduced and zero tillage was an s shaped time dependent process which reached a steady stage to the entire profile after a long-term continuous practice only.

The combined effect of all three principles like minimum soil disturbance, permanent soil cover, crop diversification was involved in the present study. Minimum disturbance of soil after the rice *ie.*, before planting of okra and before planting of cowpea resulted in better carbon sequestration. Similar result was reported by Haddaway et al., (2017) that the transition of tilled crop lands to no tillage and conservation tillage has been credited with substantial potential to mitigate climate change *via* carbon storage. This practices aim at maintaining organic matter on the surface or in the upper soil layer there by increasing soil organic carbon concentration especially in the top soil. Second principle of permanent soil cover was done by growing cowpea as *insitu* green manure and as brown manure in rice, and for okra, cowpeas green manure and crop residue mulch. The decomposition of this plant material to simple carbon compounds and assimilation and repeating cycling of carbon through the microbial biomass with formation of new cells may enhance the carbon storage. Cover crops provide soil organic carbon sequestration by increasing the input of plant residue. So the decrease of residue carbon input might be the cause of lowering the carbon sequestration in control. This results agree with the findings of Yan et al., 2011 that the long-term fertilizer experiments suggest that the fertilizer application significantly influence soil organic carbon contents in paddy field and the addition of green manure or straw may be an effective way for enhancing the carbon storage. The third principle crop diversification was followed in rice-okra-green manure system. That might be a reason for more carbon stock and sequestration in the conservation plots where the land is not kept fallow in the third season. In control treatments with out conservation practice, the land was kept fallow at third season. Negative values in control plots indicated the carbon loss and positive values in conservation treatments indicated carbon storage (Fig. 3). These results were in conformity with the findings of Vanden Bygaert et al., 2003 that the replacement of fallow with legume green manure such as lentil appeared to be an effective carbon storage potential. Similar benefits of crop rotation in sequestration was reported by Govaerts et al., 2009 that the effect of crop rotation on carbon sequestration can be due to the increase biomass carbon inputs because of the intensified production or due to the changed quality of residue input. Wani et al., 2003 reported that legume based cropping system improves the carbon sequestration.

Table 1. Effect of treatments on carbon stock (Mg ha⁻¹) (after rice, before okra) of soil

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring	22.67	27.89 ^{cd}	25.28 ^{bc}	20.84 ^a	21.86 ^{cd}	21.35 ^{cd}	17.51 ^a	16.30 ^{abc}	16.90 ^b
T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch	25.31	35.73 ^a	30.52 ^a	17.57 ^{bc}	29.16 ^a	23.36 ^{ab}	17.46 ^a	15.62 ^{cd}	16.54 ^b
T3-Direct seeding rice in flat bed + Green manuring - okra + green manuring	25.30	26.21 ^{cd}	25.76 ^{bc}	14.61 ^f	26.33 ^b	20.47 ^d	14.96 ^c	14.63 ^{de}	14.80 ^c
T4-Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch	25.84	34.35 ^{ab}	30.10 ^a	15.46 ^{df}	29.95 ^a	22.70 ^{bc}	14.77 ^c	14.59 ^{de}	14.68 ^c
T5-Direct seeding rice in raised bed + Brown manuring - okra + green manuring	19.34	24.01 ^d	21.68 ^c	17.00 ^{cde}	22.68 ^c	19.84 ^{de}	16.53 ^b	16.15 ^{bc}	16.34 ^b
T6-Direct seeding rice in raised bed + Brown manuring -okra+ crop residue mulch	23.16	27.62 ^{cd}	25.39 ^{bc}	17.19 ^{cd}	24.25 ^{bc}	20.72 ^d	16.43 ^b	16.14 ^{bc}	16.28 ^b
T7-Direct seeding rice in raised bed + Green manuring - okra + green manuring	25.93	30.17 ^{bc}	28.05 ^{ab}	19.76 ^a	28.93 ^a	24.34 ^a	17.98 ^a	17.28 ^{ab}	17.63 ^a
T8-Direct seeding rice in raised bed + Green manuring - okra+ crop residue mulch	25.35	29.48 ^{bc}	27.41 ^{ab}	19.25 ^{ab}	29.35 ^a	24.30 ^a	17.91 ^a	17.41 ^a	17.66 ^a
T9-Direct seeding rice in flat bed – okra	17.66	27.35 ^{cd}	22.51 ^c	15.03 ^{ef}	19.68 ^d	17.36 ^f	14.51 ^c	13.88 ^e	14.20 ^c
T10-Direct seeding rice in raised bed – okra	19.44	25.45 ^{cd}	22.44 ^c	15.31 ^{def}	22.08 ^{cd}	18.69 ^{ef}	14.82 ^c	14.11 ^e	14.47 ^c
SEm	-	1.18	0.99	0.68	1.19	0.75	0.44	0.40	0.41
CD(0.05)	NS	4.98	4.19	1.98	2.50	1.59	0.75	1.17	0.71

In a column, means followed by common letters do not differ significantly at 5% level by DMRT

Table 2. Effect of treatments on carbon stock (Mg ha⁻¹) (after okra, before cowpea) of soil

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring - cowpea	25.85 ^{bc}	27.87 ^{cd}	26.86 ^{cd}	23.30 ^{abc}	24.86 ^{bc}	24.08 ^{bcd}	19.67 ^a	18.78 ^a	19.23 ^a
T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch -cowpea	33.56 ^a	35.70 ^a	34.63 ^a	27.21 ^a	30.23 ^a	28.72 ^a	18.34 ^{ab}	17.95 ^a	18.15 ^a
T3-Direct seeding rice in flat bed + Green manuring - okra + green manuring - cowpea	25.47 ^{bc}	30.07 ^{bc}	27.77 ^c	25.23 ^{abc}	25.99 ^b	25.61 ^{abc}	15.31 ^d	14.74 ^{cd}	15.02 ^{bc}
T4-Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch -cowpea	28.44 ^{ab}	33.02 ^{ab}	30.73 ^b	26.74 ^{ab}	26.43 ^b	26.58 ^{ab}	17.46 ^{bc}	16.70 ^{abc}	17.08 ^{ab}
T5-Direct seeding rice in raised bed + Brown manuring - okra + green manuring -cowpea	22.51 ^{cd}	25.64 ^{de}	24.07 ^{de}	21.26 ^{cde}	21.63 ^{cd}	21.45 ^{de}	15.43 ^{cd}	15.17 ^{bcd}	15.30 ^{bc}
T6-Direct seeding rice in raised bed + Brown manuring -okra+ crop residue mulch-cowpea	24.79 ^{bcd}	29.14 ^{cd}	26.97 ^{cd}	21.62 ^{cd}	23.51 ^{bcd}	22.56 ^{cd}	17.66 ^{ab}	17.34 ^{ab}	17.50 ^a

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled
T7-Direct seeding rice in raised bed + Green manuring - okra + green manuring -cowpea	22.62 ^{cd}	25.90 ^d	24.26 ^d	22.29 ^{bc}	21.87 ^{cd}	22.08 ^d	17.61 ^{ab}	17.73 ^a	17.67 ^a
T8-Direct seeding rice in raised bed + Green manuring - okra+ crop residue mulch -cowpea	26.16 ^{bc}	27.23 ^{cd}	26.69 ^{cd}	23.83 ^{abc}	24.24 ^{bc}	24.03 ^{bcd}	18.76 ^{ab}	19.06 ^a	18.91 ^a
T9-Direct seeding rice in flat bed – okra-cowpea	17.01 ^e	16.65 ^f	16.83 ^f	16.88 ^e	16.37 ^e	16.63 ^f	14.16 ^d	13.50 ^d	13.83 ^c
T10-Direct seeding rice in raised bed – okra-cowpea	20.00 ^{de}	22.34 ^e	21.17 ^e	17.31 ^{de}	20.26 ^d	18.79 ^{ef}	14.48 ^d	13.55 ^d	14.01 ^c
SEm	1.44	1.69	1.55	1.10	1.20	1.14	0.60	0.66	0.62
CD(0.05)	5.20	3.51	2.91	4.60	3.31	3.15	2.08	2.52	2.19

In a column, means followed by common letters do not differ significantly at 5% level by DMRT

Table 3. Effect of treatments on soil carbon stock (Mg ha⁻¹) after the experiment

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled
T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring - cowpea	27.26 ^{bc}	29.59 ^b	28.42 ^c	24.70 ^b	25.59 ^b	25.15 ^c	16.94 ^{abc}	16.85 ^{abc}	16.89 ^{bc}
T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch -cowpea	35.83 ^a	36.31 ^a	36.07 ^a	30.34 ^a	28.73 ^a	29.54 ^a	18.09 ^{ab}	18.28 ^a	18.19 ^a
T3-Direct seeding rice in flat bed + Green manuring - okra + green manuring - cowpea	29.54 ^b	34.80 ^a	32.17 ^b	25.40 ^b	30.02 ^a	27.71 ^b	14.70 ^{de}	15.68 ^c	15.19 ^d
T4-Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch -cowpea	36.02 ^a	37.42 ^a	36.72 ^a	29.04 ^a	30.12 ^a	29.58 ^a	17.13 ^{abc}	18.33 ^a	17.73 ^{ab}
T5-Direct seeding rice in raised bed + Brown manuring - okra + green manuring -cowpea	23.10 ^d	28.18 ^b	25.64 ^d	21.90 ^{cd}	23.70 ^b	22.80 ^{de}	15.08 ^{de}	15.74 ^c	15.41 ^d
T6-Direct seeding rice in raised bed + Brown manuring -okra+ crop residue mulch-cowpea	28.22 ^{bc}	29.50 ^b	28.86 ^c	22.06 ^{cd}	23.94 ^b	23.00 ^{de}	15.77 ^{cd}	16.49 ^{bc}	16.13 ^{cd}
T7-Direct seeding rice in raised bed + Green manuring - okra + green manuring -cowpea	25.46 ^{cd}	28.93 ^b	27.22 ^{cd}	20.09 ^d	23.89 ^b	21.99 ^e	16.70 ^{bc}	15.53 ^c	16.11 ^{cd}
T8-Direct seeding rice in raised bed + Green manuring - okra+ crop residue mulch -cowpea	28.40 ^{bc}	29.64 ^b	29.02 ^c	22.51 ^c	24.38 ^b	23.44 ^d	18.24 ^a	17.95 ^{ab}	18.09 ^a
T9-Direct seeding rice in flat bed – okra-cowpea	16.17 ^e	17.66 ^c	16.91 ^e	13.67 ^e	13.81 ^c	13.74 ^f	13.26 ^f	13.22 ^d	13.24 ^e
T10-Direct seeding rice in raised bed – okra-cowpea	18.06 ^c	19.89 ^c	18.98 ^e	14.12 ^e	14.89 ^c	14.51 ^f	13.77 ^{ef}	13.63 ^d	13.70 ^e
SEm	2.06	2.03	2.03	1.74	1.78	1.74	0.55	0.57	0.55
CD(0.05)	3.44	3.06	2.67	1.98	2.10	1.38	1.43	1.51	1.07

In a column, means followed by common letters do not differ significantly at 5% level by DMRT

Table 4. Effect of treatments on carbon sequestration (Mg ha⁻¹) (after rice, before okra) of soil

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring	-3.88	1.34 ^{cd}	-1.27 ^{bc}	-2.67 ^a	-1.65 ^{cd}	-2.16 ^{cd}	-0.13 ^a	-1.34 ^{abc}	-0.74 ^a
T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch	-1.24	9.18 ^a	3.97 ^a	-5.95 ^{bc}	5.65 ^c	-0.15 ^{ab}	-1.02 ^b	-2.02 ^{cd}	-1.52 ^b
T3-Direct seeding rice in flat bed + Green manuring - okra + green manuring	-1.25	-0.34 ^{cd}	-0.79 ^{bc}	-8.90 ^f	2.82 ^b	-3.04 ^d	-3.52 ^d	-3.01 ^{de}	-3.26 ^c
T4-Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch	-0.71	7.80 ^{ab}	3.55 ^a	-8.06 ^{def}	6.44 ^a	-0.81 ^{bc}	-3.71 ^d	-3.05 ^{de}	-3.38 ^c
T5-Direct seeding rice in raised bed + Brown manuring - okra + green manuring	-7.21	-2.54 ^d	-4.87 ^c	-6.51 ^{cde}	-0.84 ^c	-3.67 ^{de}	-1.95 ^c	-1.49 ^{bc}	-1.72 ^b
T6-Direct seeding rice in raised bed + Brown manuring -okra+ crop residue mulch	-3.39	1.07 ^{cd}	-1.16 ^{bc}	-6.32 ^{cd}	0.74 ^{bc}	-2.79 ^d	-2.05 ^c	-1.50 ^{bc}	-1.77 ^b
T7-Direct seeding rice in raised bed + Green manuring - okra + green manuring	-0.62	3.62 ^{bc}	1.50 ^{ab}	-3.76 ^a	5.42 ^a	0.83 ^a	-0.51 ^{ab}	-0.36 ^{ab}	-0.43 ^a
T8-Direct seeding rice in raised bed + Green manuring - okra+ crop residue mulch	-1.20	2.93 ^{bc}	0.86 ^{ab}	-4.27 ^{ab}	5.84 ^a	0.79 ^a	-0.57 ^{ab}	-0.23 ^a	-0.40 ^a
T9-Direct seeding rice in flat bed – okra	-8.89	0.80 ^{cd}	-4.04 ^c	-8.48 ^{ef}	-3.83 ^d	-6.16 ^f	-3.97 ^d	-3.76 ^e	-3.86 ^c
T10-Direct seeding rice in raised bed – okra	-7.11	-1.10 ^{cd}	-4.11 ^c	-8.20 ^{def}	-1.43 ^{cd}	-4.82 ^{ef}	-3.66 ^d	-3.53 ^e	-3.59 ^c
SEm	-	1.18	0.99	0.68	1.19	0.75	0.48	0.40	0.43
CD(0.05)	NS	4.98	4.19	1.98	2.50	1.59	0.75	1.17	0.74

In a column, means followed by common letters do not differ significantly at 5% level by DMRT

Table 5. Effect of treatments on carbon sequestration (Mg ha⁻¹) (after okra, before cowpea) of soil

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring - cowpea	-0.70 ^{bc}	1.32 ^{cd}	0.31 ^{cd}	-0.21 ^{abc}	1.35 ^{bc}	0.57 ^{bcd}	2.04 ^a	1.14 ^a	1.59 ^a
T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch - cowpea	7.01 ^a	9.15 ^a	8.08 ^a	3.70 ^a	6.72 ^a	5.21 ^a	0.70 ^{ab}	0.31 ^a	0.51 ^a
T3-Direct seeding rice in flat bed + Green manuring - okra + green manuring - cowpea	-1.08 ^{bc}	3.52 ^{bc}	1.22 ^c	1.72 ^{abc}	2.48 ^b	2.10 ^{abc}	-2.33 ^d	-2.90 ^{cd}	-2.62 ^{bc}
T4-Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch - cowpea	1.89 ^{ab}	6.47 ^{ab}	4.18 ^b	3.23 ^{ab}	2.92 ^b	3.07 ^{ab}	-0.18 ^{bc}	-0.94 ^{abc}	-0.56 ^{ab}
T5-Direct seeding rice in raised bed + Brown manuring - okra + green manuring - cowpea	-4.04 ^{cd}	-0.91 ^{de}	-2.48 ^{de}	-2.25 ^{cde}	-1.88 ^{cd}	-2.07 ^{de}	-2.21 ^{cd}	-2.47 ^{bcd}	-2.34 ^{bc}
T6-Direct seeding rice in raised bed + Brown manuring -okra+ crop residue mulch-	-1.76 ^{bcd}	2.59 ^{cd}	0.42 ^{cd}	-1.89 ^{cd}	-0.08 ^{bcd}	-0.95 ^{cd}	0.02 ^{ab}	-0.30 ^{ab}	-0.14 ^a

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled
cowpea									
T7-Direct seeding rice in raised bed + Green manuring - okra + green manuring - cowpea	-3.93 ^{cd}	-0.65 ^d	-2.29 ^d	-1.22 ^{bc}	-1.65 ^{cd}	-1.43 ^d	-0.04 ^{ab}	0.09 ^a	0.03 ^a
T8-Direct seeding rice in raised bed + Green manuring - okra+ crop residue mulch - cowpea	-0.38 ^{bc}	0.68 ^{cd}	0.14 ^{cd}	0.32 ^{abc}	0.73 ^{bc}	0.53 ^{bcd}	1.12 ^{ab}	1.42 ^a	1.27 ^a
T9-Direct seeding rice in flat bed – okra-cowpea	-9.54 ^e	-9.90 ^f	-9.72 ^f	-6.63 ^e	-7.14 ^e	-6.88 ^f	-3.48 ^d	-4.14 ^d	-3.81 ^e
T10-Direct seeding rice in raised bed – okra-cowpea	-6.55 ^{de}	-4.21 ^e	-5.38 ^e	-6.20 ^{de}	-3.25 ^d	-4.72 ^{ef}	-3.16 ^d	-4.10 ^d	-3.63 ^e
SEm	1.44	1.69	1.55	1.11	1.20	1.14	0.60	0.66	0.63
CD(0.05)	5.20	3.51	2.91	4.60	3.32	3.15	2.08	2.52	2.19

In a column, means followed by common letters do not differ significantly at 5% level by DMRT

Table 6. Effect of treatments on soil carbon sequestration (Mg ha⁻¹) after the experiment

Treatments	15 cm depth			30 cm depth			45 cm depth		
	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled
T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring - cowpea	0.70 ^{bc}	3.04 ^b	1.87 ^c	1.19 ^b	2.08 ^b	1.64 ^c	-0.70 ^{abc}	-0.79 ^{abc}	-0.75 ^{bc}
T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch - cowpea	9.28 ^a	9.76 ^a	9.52 ^a	6.83 ^a	5.22 ^a	6.03 ^a	0.45 ^{ab}	0.64 ^a	0.55 ^a
T3-Direct seeding rice in flat bed + Green manuring - okra + green manuring - cowpea	2.99 ^b	8.25 ^a	5.62 ^b	1.89 ^b	6.51 ^a	4.20 ^b	-2.94 ^{de}	-1.96 ^c	-2.45 ^d
T4-Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch - cowpea	9.47 ^a	10.87 ^a	10.17 ^a	5.53 ^a	6.61 ^a	6.07 ^a	-0.51 ^{abc}	0.69 ^a	0.09 ^{ab}
T5-Direct seeding rice in raised bed + Brown manuring - okra + green manuring - cowpea	-3.45 ^d	1.63 ^b	-0.91 ^d	-1.61 ^{cd}	0.19 ^b	-0.71 ^{de}	-2.56 ^{de}	-1.90 ^c	-2.23 ^d
T6-Direct seeding rice in raised bed + Brown manuring -okra+ crop residue mulch-cowpea	1.67 ^{bc}	2.95 ^b	2.31 ^c	-1.45 ^{cd}	0.43 ^b	-0.51 ^{de}	-1.87 ^{cd}	-1.15 ^{bc}	-1.51 ^{cd}
T7-Direct seeding rice in raised bed + Green manuring - okra + green manuring - cowpea	-1.10 ^{cd}	2.44 ^b	0.67 ^{cd}	-3.42 ^d	0.38 ^b	-1.52 ^e	-0.94 ^{bc}	-2.11 ^c	-1.53 ^{cd}
T8-Direct seeding rice in raised bed + Green manuring - okra+ crop residue mulch - cowpea	1.85 ^{bc}	3.09 ^b	2.47 ^c	-1.00 ^c	0.87 ^b	-0.07 ^d	0.60 ^a	0.31 ^{ab}	0.45 ^a
T9-Direct seeding rice in flat bed – okra-cowpea	-10.38 ^e	-8.89 ^c	-9.64 ^e	-9.84 ^e	-9.71 ^c	-9.78 ^f	-4.38 ^f	-4.42 ^d	-4.40 ^e
T10-Direct seeding rice in raised bed – okra-cowpea	-8.49 ^e	-6.66 ^c	-7.58 ^e	-9.39 ^e	-8.62 ^c	-9.00 ^f	-3.87 ^{ef}	-4.01 ^d	-3.94 ^e
SEm	2.07	2.03	2.03	1.74	1.78	1.74	0.55	0.57	0.54
CD(0.05)	3.44	3.06	2.68	1.98	2.10	1.38	1.42	1.51	1.07

In a column, means followed by common letters do not differ significantly at 5% level by DMRT

Table 7. Effect of treatments on biological properties of soil after the experiment

Treatments	Microbial biomass carbon ($\mu\text{g g}^{-1}$)			Dehydrogenase activity ($\mu\text{g TPF g}^{-1} \text{soilday}^{-1}$)		
	1 st year	2 nd year	Pooled	1 st year	2 nd year	Pooled
T1- Direct seeding rice in flat bed + Brown manuring - okra+ green manuring -cowpea	115.33 ^a	127.67 ^{bc}	121.50 ^b	53.52 ^{ab}	56.18 ^b	55.82 ^b (54.86)
T2-Direct seeding rice in flat bed + Brown manuring - okra + crop residue mulch -cowpea	101.33 ^c	168.33 ^a	134.83 ^a	54.03 ^{ab}	60.29 ^a	58.71 ^a (57.17)
T3-Direct seeding rice in flat bed + Green manuring - okra + green manuring -cowpea	103.67 ^{bc}	119.33 ^{bcd}	111.50 ^c	53.81 ^{ab}	56.00 ^{bc}	55.80 ^b (54.91)
T4-Direct seeding rice in flat bed + Green manuring - okra + crop residue mulch -cowpea	109.67 ^{ab}	129.33 ^b	119.50 ^b	55.03 ^a	59.30 ^a	58.40 ^a (57.17)
T5-Direct seeding rice in raised bed + Brown manuring - okra + green manuring -cowpea	99.67 ^c	107.67 ^d	103.67 ^{cd}	51.66 ^b	55.30 ^{bcd}	54.58 ^c (53.48)
T6-Direct seeding rice in raised bed + Brown manuring -okra+ crop residue mulch-cowpea	91.67 ^d	107.33 ^d	99.50 ^d	52.15 ^b	52.90 ^e	53.17 ^d (52.53)
T7-Direct seeding rice in raised bed + Green manuring - okra + green manuring -cowpea	97.00 ^{cd}	116.33 ^{cd}	106.67 ^{cd}	53.15 ^{ab}	54.77 ^{cd}	54.74 ^{bc} (53.96)
T8-Direct seeding rice in raised bed + Green manuring - okra+ crop residue mulch -cowpea	96.73 ^{cd}	114.67 ^d	105.70 ^{cd}	52.33 ^b	54.33 ^d	54.17 ^{cd} (53.33)
T9-Direct seeding rice in flat bed – okra - fallow	77.67 ^e	71.67 ^e	74.67 ^e	42.33 ^d	45.33 ^e	44.74 ^f (43.83)
T10-Direct seeding rice in raised bed – okra - fallow	81.00 ^e	78.33 ^e	79.67 ^e	47.00 ^c	48.62 ^f	48.54 ^e (47.81)
SEm	3.69	8.53	5.78	1.23	1.43	1.35
CD(0.05)	7.62	12.02	7.90	2.44	1.30	1.21

* R (weighted MSE) transformed values, original values in parentheses.

In a column, means followed by common letters do not differ significantly at 5% level by DMRT

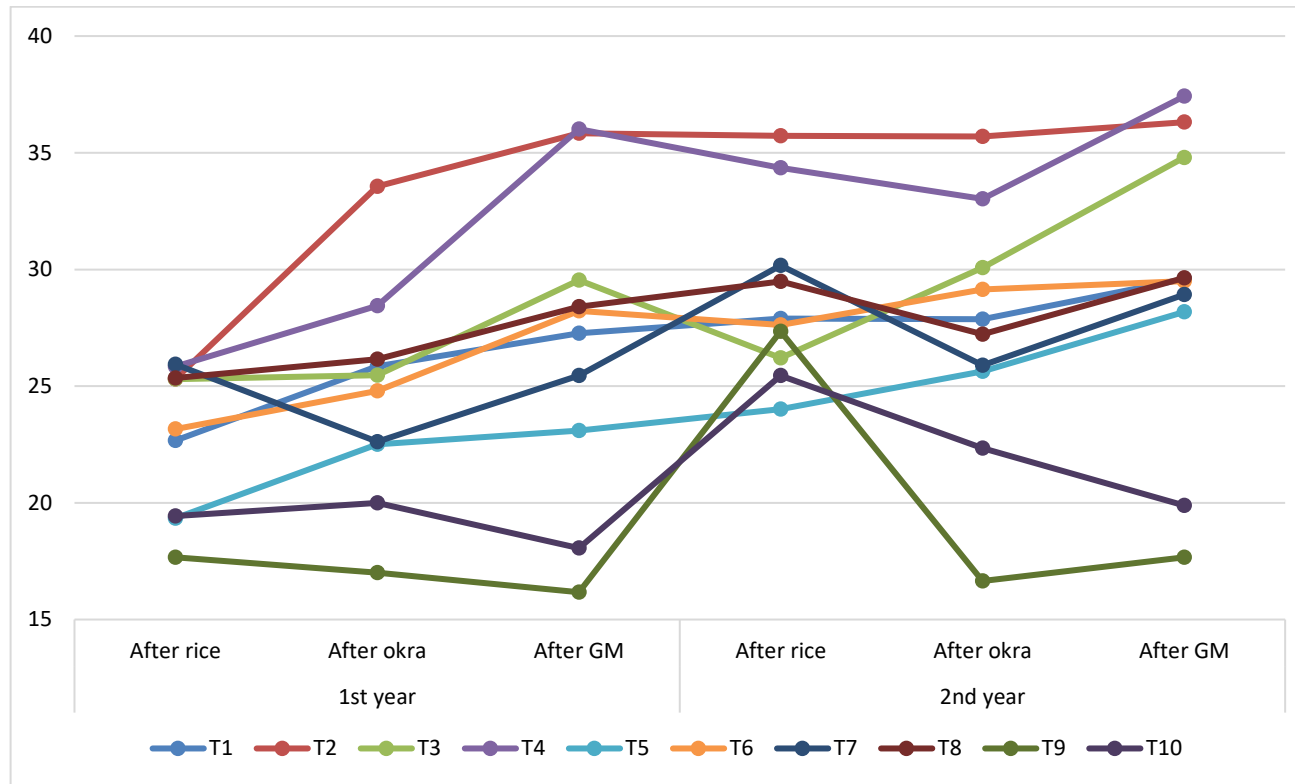


Fig. 1. Change in soil carbon stock (Mg ha⁻¹) at 15 cm depth of soil after each cropping season

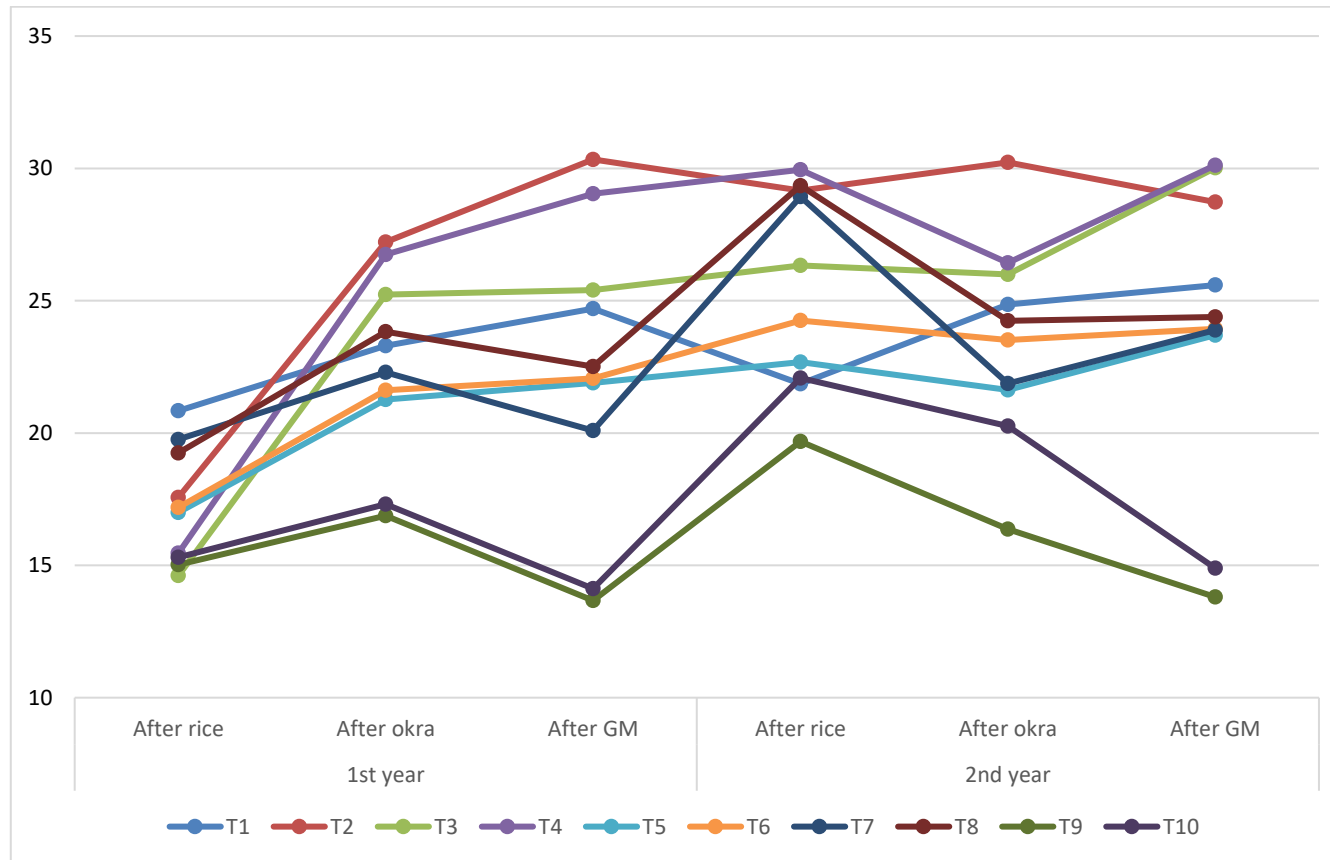


Fig 2. Change in soil carbon stock (Mg ha⁻¹) at 30 cm depth of soil after each cropping season

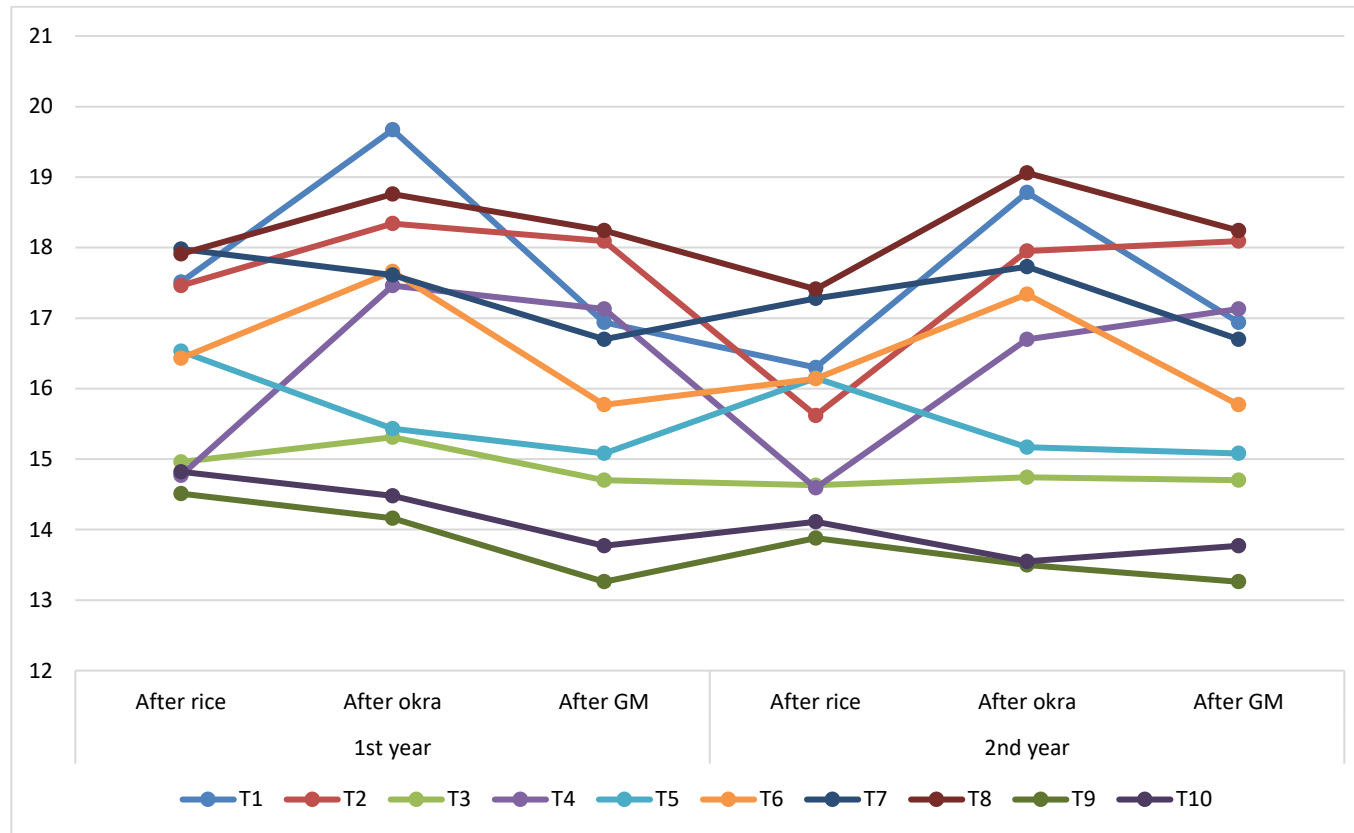


Fig 3. Change in soil carbon stock (Mg ha⁻¹) at 45cm depth of soil after each cropping season

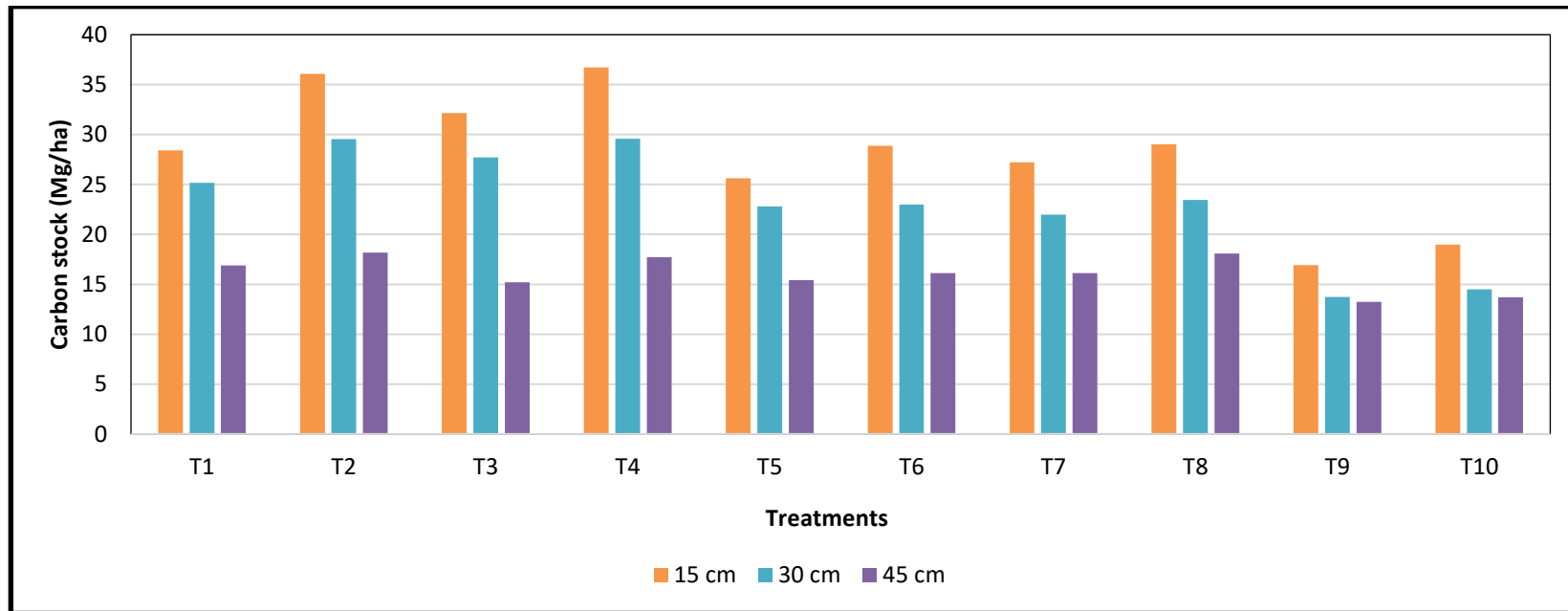


Fig. 4. Effect of conservation treatments on carbon stock of soil after the experiment

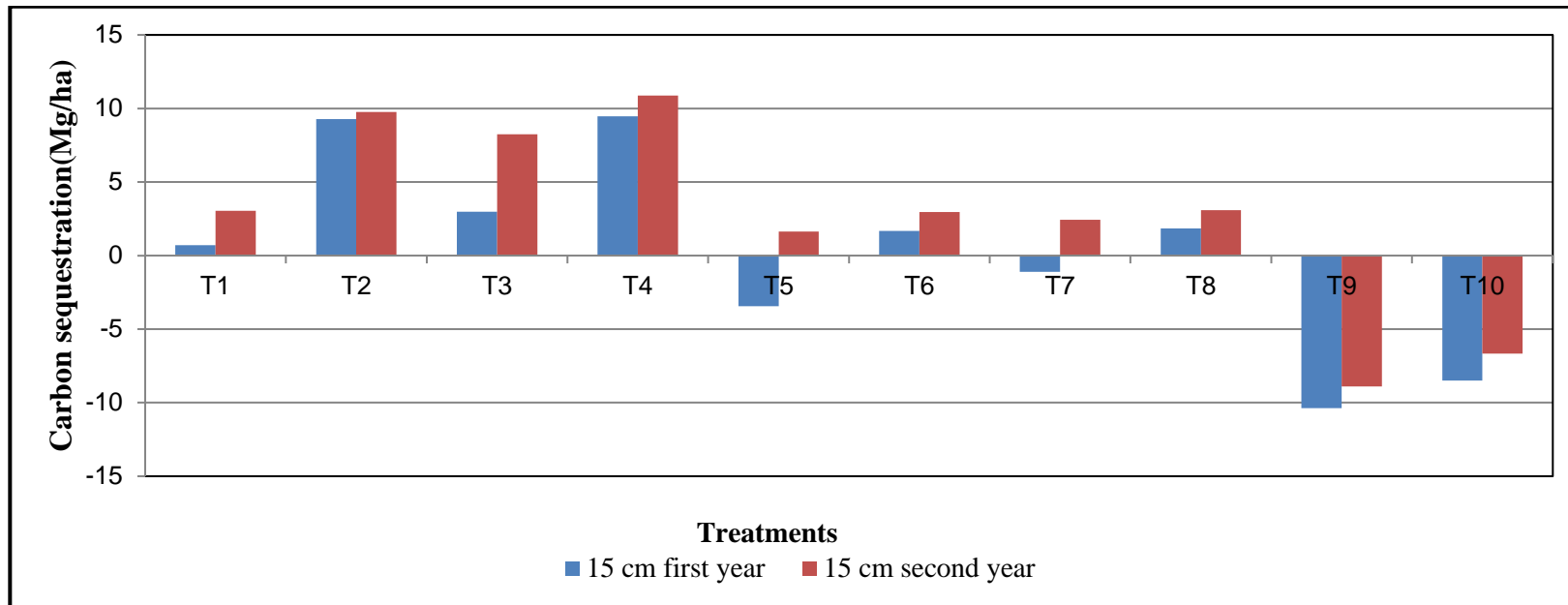


Fig. 5. Effect of conservation treatments on carbon sequestration (Mg/ha) on both the years after the experiment at 15 cm depth

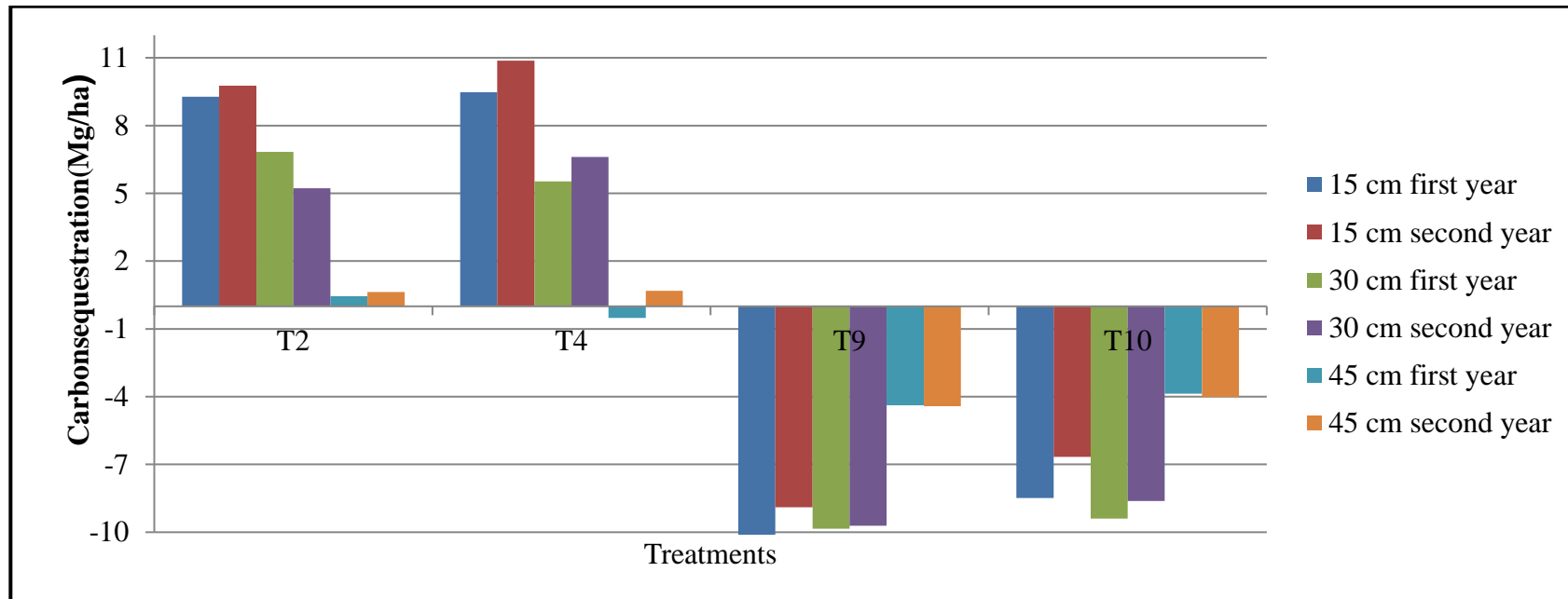


Fig. 6. Comparison of carbon sequestration (Mg/ha) for best treatments vs control in both the years after the experiment

3.3 Soil Microbial Biomass Carbon

Soil biological attributes are early warners of soil degradation and good indicators for comparing the relative performance of different soil management practices. Soil microbial biomass is the living component of the soil organic matter excluding macro fauna and plant roots. It is a well-established parameter to monitor the soil biological changes. Results of the study indicated that during both the years there was a significant influence of conservation treatments on microbial biomass carbon after the experiment (Table 7). Soil microbial biomass carbon was found to be highest in direct seeding rice in flat bed + brown manuring - okra + crop residue mulch-cowpea (T2) ($134.83 \mu\text{g g}^{-1}$) followed by direct seeding rice in flat bed + brown manuring-okra+green manuring-cowpea (T1) of $121.50 \mu\text{g g}^{-1}$ and it in turn was on par with direct seeding rice in flat bed + green manuring-okra+crop residue mulch-cowpea (T4) ($119.50 \mu\text{g g}^{-1}$). Direct seeding rice in flat bed-okra-fallow (T9) registered the lowest value of $74.67 \mu\text{g g}^{-1}$ followed by direct seeding rice in raised bed –okra –fallow ($79.67 \mu\text{g g}^{-1}$) and they both are on par to each other. Direct seeding rice in flat bed + brown manuring - okra + crop residue mulch – cowpea recorded the highest microbial biomass carbon. For brown manuring in rice 2-4-D was used as herbicide and for the degradation of herbicide the microbial population may increase and thereby SMBC might have improved. Similar results were reported by Ramalakshmi et al., (2017) that the herbicides at the recommended rate in rice decreased the soil microbial population initially and after that microbial population was increased due to the degradation of herbicide, which acted as a nutrient source for the growth of microorganisms. Basilla, (2020) also reported that the SMBC was higher under brown manuring on 25 DAS and pyrazosulfuron-ethyl on 6 DAS. Minimum tillage might also be a reason for an increase in SMBC in conservation practices than in the conventional method. Similar results were reported by Lal & Jacinthe, (2009), 2009; Ma et al., 2016 that conservation tillage increased SMBC than conventional. The conventional practice of direct seeding rice in flat bed - okra– fallow recorded the lowest SMBC and it might be due to lack of nutrient source for microbial growth.

3.4 Dehydrogenase Activity

Dehydrogenase activity is a valued bio marker of soil quality indicator, which is strongly correlated with the organic carbon present in the soil and soil microbial biomass. It can be used as a tool for assessing the sustainability of an agricultural ecosystem. Significantly higher dehydrogenase activity was in direct seeding rice in flat bed + brown manuring - okra + crop residue mulch-cowpea followed by direct seeding rice in flat bed + green manuring –okra + crop residue mulch-cowpea and recorded 23.33 % higher dehydrogenase activity than in the conventional practice (Table 7). Dehydrogenase activity was found to be significantly higher in direct seeding rice in flat bed + brown manuring - okra + crop residue mulch-cowpea ($57.17 \mu\text{g TPF g}^{-1} \text{soilday}^{-1}$) and it was on par with direct seeding rice in flat bed + green manuring –okra + crop residue mulch-cowpea ($57.17 \mu\text{g TPF g}^{-1} \text{soilday}^{-1}$). Direct seeding rice in flat bed-okra-fallow (T9) registered the lowest dehydrogenase activity of $43.83 \mu\text{g TPF g}^{-1} \text{soilday}^{-1}$ followed by direct seeding rice in raised bed –okra –fallow ($47.81 \mu\text{g TPF g}^{-1} \text{soilday}^{-1}$). The increase in the enzyme activity might be due to an increase in soil organic carbon content by microbial decomposition of organic matter. A similar result was reported by Rajanand, (2018), that the dehydrogenase activity increased with an increase in soluble organic carbon content in the soil with a corresponding increase in the degrading soil biota. The addition of cowpea either as green manure or brown manure and crop residue triggered the activity of dehydrogenase due to increased organic carbon. Minimum soil disturbance also might be a reason for the improvement of enzyme activity. The lowest dehydrogenase activity was reported in direct seeding rice in flat bed-okra-fallow it might be due to less soil microbial activity, soil moisture and organic carbon. The results are in conformity with the findings of Rajanand (2018) that the lack of sufficient organic carbon and soil moisture might have hindered the activity and multiplication of soil biota in rice-rice-fallow system, which in turn might have reduced the enzyme activity.

4. Conclusion

Rice - okra- green manure cropping system with conservation practice had a significant influence on carbon stock, soil carbon sequestration and soil biological properties compared with Rice - okra cropping system without conservation practices. The highest soil carbon stock and sequestration at 15 and 30 cm was recorded in direct seeding rice in flat bed + green manuring- okra+ crop residue mulch –cowpea. Indirect seeding rice in flat bed - okra-fallow with out conservation agriculture practices showed a negative value for sequestration. A slow increase in sequestration after each crop of this study clearly indicated that conservation agriculture practices for a long run will sequester the carbon to entire soil profile. Soil biological properties like microbial biomass

carbon and dehydrogenase activity were significantly improved by conservation agriculture practices. Long term conservation agriculture practices in upland rice–vegetable cropping system by applying the three principles such as minimum soil disturbance, permanent soil cover and crop diversification by the elimination of fallow can be recommended as a viable option to increase the carbon sequestration and to mitigate the climate change.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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