Long-term effect of tillage, residue and biofertilizer on soil microbial biomass carbon and dehydrogenase activity under rice-wheat cropping system in Terai agro-ecological region of West Bengal

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ABSTRACT

As a major supply of staple foods, the rice-wheat cropping system is crucial to global food security. But several soil environmental problems arise due to this continuous cultivation of these crops. This problem includes the decline of soil health and quality day by day. In this situation, maintaining soil health requires conservation agriculture. In this context, we assessed the effect of tillage, residue and biofertilizers on soil dehydrogenase activity (SDHA) and soil microbial biomass carbon (SMBC) of post wheat soils (0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm) which were cultivated since 2006 as long term field experiment in rice-wheat cropping system in factorial RBD with three replications in Terai agro-ecological regions of west Bengal. Results revealed that all the SDHA and SMBC were increased by the influence of long-term zero tillage, bio fertilizers & residue addition in the most of the soils depths under study except 20-40 cm. Highest short-term changes of 213.19 % as compared to the initial soils of SDHA was observed in conventionally tilled soil with biofertilizer addition treatment combinations. As these parameters are key important soil health & quality determinant, here in this context we tried to establish the relationship between SDHA and SMBC. A positive good regression coefficient of 0.74, 0.65 and 0.67 in 0-5 cm, 5-10 cm and 10-20 cm respectively was observed between the two parameters in all the soil depths under study. With growing future demand of foods, conservation agriculture should be practiced to maintain the sustainability of soil productivity in long term basis. It is evidenced in our study that these major soil health parameters increased where zero tillage was practiced along with the residue and biofertilizer addition.

Keywords: Conservation Agriculture, Zero Tillage, soil biological properties, soil health

1. INTRODUCTION

The rice-wheat cropping system is of significant importance in ensuring global food security, as it serves as a primary source of staple foods for the world's population (Lalik et al., 2014; Banjara et al., 2021). In the Asian continent, a total of 13.5 million hectares are dedicated to agricultural cultivation, with the majority, around 57%, being in the South Asian region (Ahmad and Iram, 2006). The rice-wheat cropping system, which is the primary production system in India, has a vast land area of 9.2 million hectares, making it a crucial contributor to the country's food security (Jat et al., 2020). But continued RWCS implementation in India has led to significant problems resulting into a decline in the system's productivity. Major threats to its sustainability include the depletion of the soil's nutrient pool, declining soil health, groundwater depletion, rising production costs, labour shortages, environmental pollution from crop residue burning and increased greenhouse gas emissions, climatic vulnerabilities, and herbicide resistance in weed species (Dhanda et al., 2022). Rice and wheat, as cereal crops, have a significant impact on soil nutrient depletion due to their exhausting nature. This issue is further exacerbated by the practice of burning rice crop residue in fields following mechanized harvesting, exacerbating the situation. Approximately two million farmers in the northwestern region and certain areas of eastern India engage in the practice of burning an estimated 23 million metric tons of rice residue annually.

Conservation Agriculture (CA) is a sustainable farming approach centered on three core principles: minimal soil disturbance, permanent soil cover, and diversified crop rotations. This holistic approach improves soil structure, reduces erosion, conserves water, promotes beneficial organisms, and decreases the need for chemical inputs, ultimately fostering resilient and productive agricultural systems that are environmentally sustainable and economically viable. In this scenario, it is important to manage and sustain the soil health by adopting the conservation agriculture methods. The effect of tillage on soil biological health is pivotal in agricultural sustainability. While light tillage can initially stimulate microbial activity through improved aeration, intensive or frequent tillage disrupts soil structure, diminishing microbial abundance and diversity, and accelerating organic matter decomposition. Transitioning to reduced tillage, no-tillage, cover cropping, and diverse rotations can ameliorate these impacts, preserving soil structure, fostering microbial communities, and sustaining long-term soil health, ultimately enhancing agricultural productivity and ecological equilibrium. The management of crop residues and biofertilizers significantly influences soil biological properties. Crop residues serve as a substrate for microbial growth, providing a continuous source of organic matter that fuels enzymatic reactions. As microorganisms decompose these residues, they release enzymes that break down complex organic compounds into simpler forms, making nutrients available for plant uptake. Biofertilizers introduce diverse microbial populations to the soil, enhancing enzymatic diversity and efficiency. Beneficial microorganisms present in biofertilizers produce enzymes that aid in nutrient cycling, organic matter decomposition, and disease suppression. Proper management of both crop residues and biofertilizers, such as incorporating residues into the soil and applying biofertilizers at optimal rates, can stimulate enzymatic activity, promoting nutrient availability, soil structure improvement, and overall soil biological health (Mahmud et al., 2021).

In this study, we hypothesized that soil enzymatic activity through the activation of microorganisms will be influenced by the long-term effect of tillage, bio-fertilizer, and residues in rice-wheat cropping system. Thus, the objective of the present study was to examine

- Soil dehydrogenase and soil microbial biomass carbon as influenced by the long-term tillage, residue and bio-fertilizer management.
- Short-term effects of treatments on soil dehydrogenase and soil microbial biomass carbon activities.

2. MATERIAL AND METHODS

2.1 Site description: A long-term filed experiment was started in 2006 with undisturbed treatment plots in University Research Farm located in Pundibari, Cooch Behar district, West Bengal (26° 24' 4.30" N, 89° 23' 11.27" E) with rice-wheat cropping system. The climate of the region is sub-tropical and per-humid. The experiment had 3 treatment factors each with two levels such as: (1) Tillage practice- zero tillage (ZT), conventional tillage (CT); (2) Crop residue crop residue addition (R_1), crop residue removal (R_0) and (3) Seed inoculated bio-fertilizer-application of bio-fertilizer (B_1), no application of bio-fertilizer (B_0). The treatment combinations are showed in table no 1. We studied some biological parameters in this research plots over a period of 2 years, of post wheat 2020 as 1st cropping season and post wheat 2021 as 2nd cropping season. The results presented in this topic reflect the cumulative effect of treatment variables on soil dehydrogenase enzymatic activity after 15-17 years of rice-wheat cropping in a long-term research field maintained at the university farm.

Table: 1. Details of treatments maintained in long-term field experiment for rice and wheat crop since 2006.

Treatments	Treatment details
T_1	Zero tillage (ZT) + Residue removed (R_0) + Bio-fertilizer (B_1)
T_2	Zero tillage (ZT) + Residue removed (R_0) + No bio-fertilizer(B_0)
T_3	Zero tillage (ZT) + Residue added (R_1) + Bio-fertilizer(B_1)
T_4	Zero tillage (ZT) + Residue added (R_1) + No bio-fertilizer (B_0)
T_5	Conventional tillage (CT) + Residue removed(R_0) + No bio-fertilizer(B_0)
T_6	Conventional tillage (CT) + Residue removed(R_0) + No bio fertilizer(B_0)
T_7	Conventional tillage (CT) + Residue added (R_0) + Bio-fertilizer (B_1)
T_8	Conventional tillage (CT) + Residue added (R_0) + No bio fertilizer(B_1)

2.2 Soil Sampling:

The soil samples utilized for analysis in this study were composite samples, consisting of sub-samples collected from each treatment plot of the experimental field at multiple depths (0-5, 5-10, 10-20, and 20-40 cm) after each harvest of the wheat crop. The soil samples were brought to the laboratory in icebox to minimize the microbial activity and kept in the refrigerator until analyzed.

2.3 Soil analysis:

2.3.1 Dehydrogenase activity:

For determining dehydrogenase, 5g field moist soil in a stopper test tube was incubated at 28^0 C for 24 hours with 1 ml 3% 2, 3, 5-triphenyl tetrazolium chloride, $CaCO_3$ and 1% glucose; soil washed with methanol and the intensity of red colour was measured at 485 nm with spectrophotometer. This quantifies microbial activity through tetrazolium reduction's colour change (Casida et al., 1964).

2.3.2 Microbial Biomass Carbon: The microbial biomass carbon (MBC) was estimated using the Chloroform Fumigation method as outlined by Jenkinson (1988). In this procedure, field soil samples were placed in a 100 ml beaker and fumigated with chloroform for 24 hours in vacuum desiccator. Following fumigation, both the fumigated and unfumigated soils were subjected to extraction with 0.5M K_2SO_4 by shaking for half an hour. The carbon content present in the filtrate was determined by titrating 0.4N $K_2Cr_2O_7$ with Fe $(NH)_2SO_4$ using DPA as the indicator. The calculation was performed considering a K_{EC} value of 0.38± 0.05.

3. RESULTS AND DISCUSSION

3.1 Effect of long-term tillage residue and bio fertilizer on soil dehydrogenase activity:
Long-term experimental fields offer an excellent opportunity to investigate the effects of different agricultural systems on soil biology, a critical factor in predicting the productivity of soil. The assessment of soil biological health, which encompasses the activity of soil enzymes, is a component of soil health and serves as a valuable indicator of the ecological attributes of the soil. The enzyme dehydrogenase is present in all living microbial cells (Watts et al., 2010). Activity of this enzyme depicts metabolic health of soil microbes. One of

the most sensitive bio-indicators of soil fertility is activity of soil dehydrogenase activity (SDHA) (Wolinska and Stepniewska, 2012).

Soil dehydrogenase activity of post wheat soils as influenced by the treatment combinations is shown in table 2. Soil dehydrogenase activity was noted a decrease with soil depth irrespective of the treatments in both years of study soils. In the 1st cropping season, highest value of 29.27 µgTPF g^{-1} 24hr^{-1} was observed in Zero tillage plot with bioinnoculations that was statistically at par with zero tillage with residue addition & bio-inoculants (T_3) and zero tillage with residue addition (T_4) in 0-5 cm soil depth. In 5-10 cm soil layer, increased SDHA was noticed in T_1, which was statically at par with all the treatments except Zero tillage without bio-inoculants and residue removed (T_2), zero tillage with residue addition (T_4) and (CT without residue and bio-inoculants) T_6. Maximum SDHA was found in T_7 both in 10-20 cm & 20-40 cm soil layers, which being 10.71 and 2.93 µgTPF g^{-1} 24hr^{-1} respectively.

In 2nd cropping season, dehydrogenase recorded into the highest value (34.80 µgTPF g^{-1} 24hr^{-1}) at 0-5 cm soil depth. However, in 5-10 cm layer, maximum recorded into highest value of 16.93 µg TPF g^{-1} 24hr^{-1} at DHA was recorded in T_1 treatment and it was significantly higher than other treatments, except T_2, T_5 and T_6 which was were statistically at per with one another. At 10-20 cm soil depth, significantly highest value of DHA (7.33 µg TPF g^{-1} 24hr^{-1}) was noticed in T_7. Significantly, highest values of 3.44 µg TPF g^{-1} 24hr^{-1} was noticed in T_2 in 20-40 cm soil depth.

Relatively higher values of dehydrogenase in surface layers and more so uppermost (0-5 cm) soil layer irrespective of tillage may be explained by the higher supply of substrate along with higher availability of oxygen and water creating conductive environment of microbial activity in uppermost layer than the lower ones with soil depth. These results are corroborated with the findings of (Kumar et al., 2018, Sahoo et al., 2023).

Table: 2 Soil dehydrogenase activity in the treatment combinations

	1st cropping season				**2nd Cropping season**			
Treatment	**0-5 cm**	**5-10 cm**	**10-20 cm**	**20-40 cm**	**0-5 cm**	**5-10 cm**	**10-20 cm**	**20-40 cm**
T_1 (ZTR_0B_1)	29.27^{a}	15.27^{a}	5.14^{bc}	2.27^{cd}	34.80^{a}	16.93^{a}	3.15^{b}	2.32^{c}
T_2 (ZTR_0B_0)	17.88^{bc}	12.90^{cd}	3.67^{bc}	2.93^{a}	18.48d^{ef}	14.50^{bcd}	2.36^{b}	3.44^{a}
T_3 (ZTR_1B_1)	26.70^{a}	14.85^{ab}	4.21^{bc}	2.35^{bc}	29.47^{b}	16.59^{a}	7.22^{a}	2.30^{c}
T_4 (ZTR_1B_0)	26.00^{a}	13.82^{bcd}	3.16^{c}	2.63^{b}	24.75^{c}	15.65^{ab}	4.05^{b}	3.07^{ab}
T_5 (CTR_0B_1)	17.44^{bc}	14.00^{abcd}	3.41^{c}	1.88^{e}	19.71^{de}	14.09^{cd}	8.20^{a}	2.34^{c}
T_6 (CTR_0B_0)	17.77^{bc}	12.74^{d}	6.48^{b}	2.05^{de}	16.32^{f}	13.67^{d}	3.30^{b}	2.55^{c}
T_7 CTR_1B_1	20.25^{b}	14.47^{ab}	10.71^{a}	2.48^{bc}	21.53d	16.12^{ab}	8.45^{a}	2.70^{bc}
T_8 CTR_1B_0	15.24^{c}	14.26^{abc}	5.94^{bc}	2.01^{de}	17.76^{ef}	15.65^{abc}	8.68^{a}	2.53^{c}
SEM	**1.09**	**0.60**	**0.85**	**0.09**	**0.98**	**0.54**	**0.75**	**0.13**
LSD ($p \leq 0.05$)	**3.30**	**1.82**	**2.58**	**0.28**	**2.96**	**1.64**	**2.28**	**0.39**

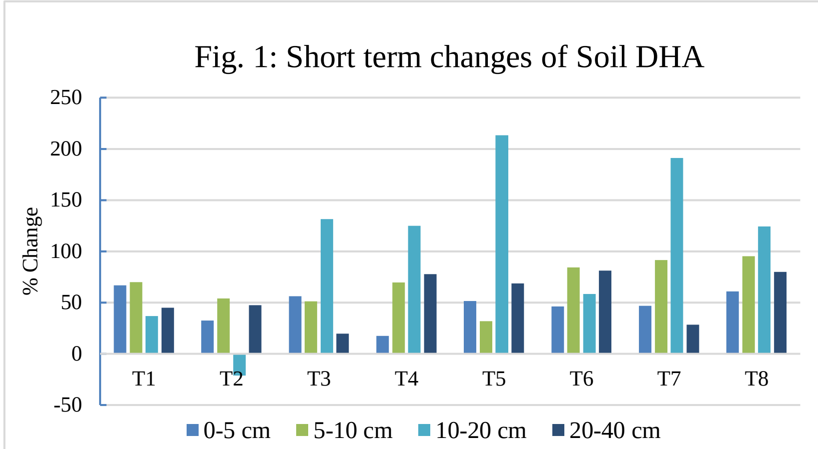
It is evidenced from the results (table-3) that in both the years soil dehydrogenase activity in zero tillage was significantly ($p \leq 0.05$) higher than the conventional tillage system in all the soil depths under study except in 5-10 cm layer in 1st cropping season. Residue addition significantly increased that SDHA in 5-10 cm soil depth in 2nd cropping season and 10- 20 cm soil depth in both cropping seasons. Biofertilizers inoculation proved to increase significantly ($p \leq 0.05$) this enzymatic activity in all the soil depths except 10- 20 cm soil depth in 1st cropping season and 20-40 cm in both cropping seasons. Interaction effect of tillage, residue and biofertilizers are found significant in 0-5 cm soil depth in both cropping season

and 10- 20 cm soil depth in in 1st cropping season. ZT practice retain crop residues and organic matter on the soil surface, providing a continual supply of organic carbon for dehydrogenase enzymes. The undisturbed soil structure in ZT soils is favorable for microorganisms responsible for dehydrogenase enzyme production. Zero tillage (ZT) practices favors by reducing soil erosion, enhancing moisture retentions & alleviating soil compactions, zero tillage practice favors nutrient cycling, faster a more conductive environment leading to increased dehydrogenase activity. Bowles et al. (2014) shown that variations in organic management practices have exerted a significant impact on soil enzyme activity, while having a minimal effect on microbial populations. Similar results are also obtained by Mijangos et al., (2006) & Burns and Dick., (2002).
Soil dehydrogenase activity is significantly affected by the kind of crop cover and the management strategies for the resulting residue. Similar increases in enzyme activity were seen in plots where crop residue or residue had been mulched; the magnitude of these increases was 156 and 147% larger, respectively, than in the residue removal plots (Singh et al., 2018). Dehydrogenase activity was significantly affected by a combination of crop rotation and residue management. Increased availability of nutrients for microbial metabolism may account for the beneficial effect of crop waste and Leucaena mulch on dehydrogenase activity (Manjaiah and Singh, 2001) in addition to providing nutrients, crop leftovers may also affect the soil's moisture, temperature, and aeration. Soil residue and mulches can affect microbial metabolism by changing the soil's microclimate (Sinsabaugh & Follstad Shah, 2011). Therefore, they noticed higher dehydrogenase and SMBC increased in residue added with biofertilizer treated plots than non-treated plots and higher in surface soil than the other layer soils.

Table 3: Main Effect and interaction effect on soil dehydrogenase activity

Treatment		**1st cropping season**				**2nd Cropping season**			
		0-5 cm	**5-10 cm**	**10-20 cm**	**20-40 cm**	**0-5 cm**	**5-10 cm**	**10-20 cm**	**20-40 cm**
	ZT	24.96	14.21	4.04	2.55	26.87	15.92	4.19	2.78
	CT	17.67	13.87	6.64	2.10	18.83	14.88	7.16	2.53
Tillage (T)	**SEM (±)**	**0.54**	**0.21**	**0.43**	**0.05**	**0.49**	**0.27**	**0.38**	**0.06**
	LSD (*P≤0.05*)	**1.65**	**NS**	**1.29**	**0.14**	**1.48**	**0.82**	**1.14**	**0.20**
	R_0	20.59	13.73	4.68	2.28	22.33	14.80	4.25	2.67
	R_1	22.05	14.35	6.00	2.37	23.38	16.00	7.10	2.65
Residue (R)	**SEM (±)**	**0.54**	**0.21**	**0.43**	**0.05**	**0.49**	**0.27**	**0.38**	**0.06**
	LSD (*P≤0.05*)	**NS**	**NS**	**1.29**	**NS**	**NS**	**0.82**	**1.14**	**NS**
	B_0	19.22	13.43	4.81	2.41	19.33	14.87	4.60	2.90
	B_1	23.41	14.65	5.87	2.24	26.38	15.93	6.75	2.42
Bio fertilizers (B)	**SEM (±)**	**0.54**	**0.21**	**0.43**	**0.05**	**0.49**	**0.27**	**0.38**	**0.06**
	LSD (*P≤0.05*)	**1.65**	**0.64**	**NS**	**0.14**	**1.48**	**0.82**	**1.14**	**0.20**
Interaction effect									
T X R		**NS**	**NS**	**1.83**	**0.20**	**NS**	**NS**	**NS**	**NS**
T X B		**2.33**	**NS**	**NS**	**0.20**	**2.09**	**NS**	**1.61**	**0.28**
R X B		**NS**	**NS**	**1.83**	**0.20**	**2.09**	**NS**	**NS**	**NS**
T X R X B		**3.30**	**NS**	**2.58**	**NS**	**2.96**	**NS**	**2.28**	**NS**

Short term change (%) of Soil DHA compared to SDHA of before start of my experiment is depicted in fig.1. In this figure, it is evidenced that there was increment of SDHA in all the soil depths and treatments except 10-20 cm depth of T_2. In 10-20 cm, soil there was maximum increase of soil DHA as compared to other soil depths except the T_1 and T_2. Short term change of Soil DHA was increased maximum (213.19 %) in 10-20 cm soil depth of T_5 which was conventionally cultivated with bio fertilizer inoculation.



3.2 Effect of long-term tillage residue and bio fertilizer on SMBC:

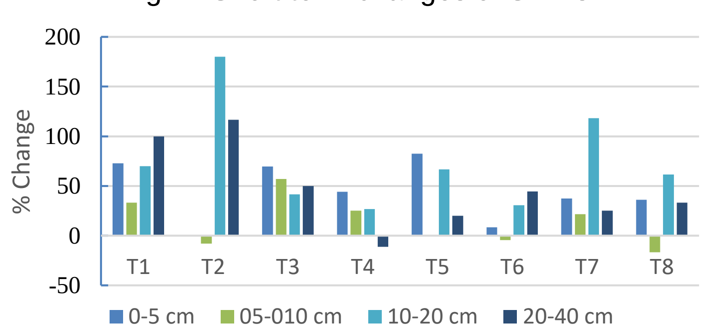
Microbial biomass carbon as influenced by the long-term tillage, residue and biofertilizers in two consecutive cropping seasons are shown in table 3. The microbial biomass is considered to be very susceptible to change and is a significant component of the organic matter pool. An elevation in microbial biomass carbon (MBC) is more likely to accurately reflect alterations in the nutrient-providing capability of organic matter compared to an increase in total organic matter. The range of MBC values as stated in Table 3 ranges from 124.44 to 764.44 mg C Kg^{-1} across various soil depths irrespective of cropping season. The findings of the study revealed a statistically significant difference ($p \leq 0.05$) in the levels of soil microbial biomass carbon (MBC) among the treatment combinations of crop residue, biofertilizers and tillage. Soil Microbial Biomass Carbon (SMBC) as influenced by the treatment's combination (Table 4). In 1^{st} cropping season, in 0-5 cm soil, highest value of 764.44 mg/Kg was noted in T_3, which was statistically at par with T_1 & T_4, and lowest value of 426.67 mg/Kg was observed in conventionally tilled without biofertilizer and residue treatment plots (T_6). The MBC values exhibited a notable increase in the treatments (T_3 and T_4) where crop residues were conserved by zero tillage methods and particularly in treatment T_3. The primary reason that contributed to the increase in MBC in T_3 includes the presence of easily metabolizable and hydrolyzable carbon and nitrogen in crop residues, as well as reduced soil disturbance due to adoption of zero tillage. Similar trend was also observed in 5-10 cm soil, lowest value of 320 mg/Kg was found both in T_2 and T_6. In the year of 2021, significant difference between the treatments was observed in 0-5 cm and 5-10 cm. In 0-5 cm soil depth, maximum value of 693.33 mg/Kg of SMBC was recorded in T_3, which was statistically at par with T_1, T_4, T_7 and T_8. Highest value of 586.67 mg/Kg was recorded in T_3 at 5-10 cm soil depth and it was statistically at par with all the treatments except T_2. T_4 and T_5. In 10-20 cm and 20-40 cm soil layers no significant difference among these treatments was observed in both the years of study. The addition of crop residue with biofertilizer was shown to have a considerable positive impact on the microbial biomass, as reported by Banerjee et al., (2006). The findings of this study emphasize the significance of incorporating crop waste and biofertilizers in no tillage practices as a means to enhance the microbial biomass carbon content in soil. However, Sharma (2001) reported a notable disparity in the levels of MBC and DHA activities between the surface and sub-surface soil. Attributing to limited presence of crop residue in the lower soil depths than the upper one. The study conducted by Franzluebbers et al. (1999) showed that variations in carbon inputs

Table 4: Soil Microbial Biomass Carbon (mg/Kg) in the treatment combinations								
	1st cropping season				2nd Cropping season			
Treatment	**0-5 cm**	**5-10 cm**	**10-20 cm**	**20-40 cm**	**0-5 cm**	**5-10 cm**	**10-20 cm**	**20-40 cm**
$T_1 (ZTR_0B_1)$	657.78^{ab}	462.22^{ab}	248.89	124.44	675.56^{a}	497.78^{a}	302.22	177.78
$T_2 (ZTR_0B_0)$	444.44^{d}	320.00^{c}	213.33	142.22	480.00^{c}	408.89^{c}	248.89	231.11
$T_3 (ZTR_1B_1)$	764.44^{a}	480.00^{a}	231.11	160.00	693.33^{a}	586.67^{a}	302.22	160.00
$T_4 (ZTR_1B_0)$	622.22^{abc}	462.22^{ab}	302.22	142.22	640.00^{ab}	533.33^{ab}	337.78	142.22
$T_5 (CTR_0B_1)$	533.33^{bcd}	391.11^{abc}	320.00	231.11	551.11^{bc}	480.00^{bc}	355.56	213.33
$T_6 (CTR_0B_0)$	426.67^{d}	320.00^{c}	213.33	177.78	462.22^{c}	373.33^{c}	302.22	231.11
T_7 CTR_1B_1	586.67^{bcd}	373.33^{bc}	373.33	160.00	586.67^{ab}	497.78^{ab}	426.67	177.78
T_8 CTR_1B_0	480.00^{cd}	391.11^{abc}	320.00	124.44	604.44^{ab}	444.44^{ab}	373.33	142.22
Sem ±	**52.91**	**30.14**	**36.26**	**49.66**	**32.57**	**32.49**	**33.85**	**53.12**
LSD ($P\leq0.05$)	**160.48**	**91.43**	**NS**	**NS**	**98.80**	**98.54**	**NS**	**NS**

to the soil lead to corresponding expansions or contractions both in the total organic carbon and the microbiological pool. Crop left overs provide a consistent and homogeneous supply of carbon, which acts as an energy source for microorganisms. The relationship between tillage and residue management has a discernible impact. The implementation of zero tillage with residue removal led to a decrease in soil microbial biomass (SMB), as seen in Table 3. Conversely, zero tillage (ZT) with residue retention demonstrated the ability to sustain a high level of SMB. These resulted resembles with the findings of Salinas-Garcia et al. (2001) in Central Mexico, where they observed that zero tillage combined with partial or full residue retention resulted in increased soil microbial biomass carbon (SMBC) compared to either conventional tillage or zero tillage but with no residue application. The accumulation of organic carbon in the topsoil is facilitated by the retention of residue on the soil surface, which in turn provides microbial substrates of varying quality and quantity. This phenomenon has an impact on the dynamics of soil carbon (C) and nutrient cycling, resulting in an increase in soil microbial biomass (SMB). The presence of crop residues on the soil surface has been found to enhance microbial abundance due to the favorable growth and multiplication favored by the mulch layer (Carter and Mele, 1992)

Short term changes of SMBC as influenced by the treatments averaged over two years of study are depicted in Fig: 2. An overall positive change of SMBC was recorded in all the treatments at all soil depths, except at 0-5 cm in T_2, 20-40 cm in T_4, 5-10 cm in T6 and 5-10 cm in T_8. Maximum increase of 180.00 % was noticed in T_2 (ZT with residue removed and no bio inoculation plot) of 5-10 cm soil depth. Maximum decrease (16.67 %) of SMBC values are noticed in 5- 10 cm in T_8 (CT with residue addition and no bio inoculated treatment plot).

Fig. 2: Short term changes of SMBC



Main effect of tillage, residue and biofertilizer on SMBC are shown in table 5. The estimate of microbial biomass carbon (MBC) is a crucial factor in distinguishing between organic and conventional

environments. (Monokrousos, Papatheodorou, and Stamou, 2008). Zero tillage and residue addition practice have proved to significantly (p≤0.05) increase Soil MBC over Conventional tilled and residue removed soils in all the soil depths except 20-40 cm soil depth in both the cropping season. Effect of biofertilizers on SMBC was confined up to the 10 cm soil depth. Biofertilizers inoculation significantly increase the soil MBC in 0-5 cm and
5-10 cm soil depth than the no bio-inoculated soils in both cropping seasons.
In Punjab, India, experiments in the Rice-wheat cropping system demonstrated that keeping crop residues in the field increased soil organic carbon (SOC) compared to their removal (Singh et al., 2000). This rise in SOC was closely linked to enzyme activity, likely due to the fact that a larger soil microbial biomass supposed by higher substrate carbon leading to increased enzyme activity. But, in 20-40 cm soil depth, there is no significant effect of tillage, residue or biofertilizer on SMBC.

Table 5: Main Effect and interaction effect on Soil Microbial Biomass Carbon (mg/Kg)

Treatment		**Post wheat 2020**				**Post wheat 2021**			
		0-5 cm	**5-10 cm**	**10-20 cm**	**20-40 cm**	**0-5 cm**	**5-10 cm**	**10-20 cm**	**20-40 cm**
	ZT	622.22	431.11	248.89	142.22	622.22	506.67	297.78	177.78
	CT	506.67	368.89	306.67	173.33	551.11	448.89	364.44	191.11
Tillage (T)	**SEM (±)**	**26.45**	**15.07**	**18.13**	**24.83**	**16.29**	**16.24**	**16.92**	**26.56**
	LSD (*P≤0.05*)	**80.24**	**45.72**	**55.00**	**NS**	**49.40**	**49.27**	**51.33**	**NS**
	R_0	515.56	373.33	248.89	168.89	542.22	440.00	302.22	213.33
	R_1	613.33	426.67	306.67	146.67	631.11	515.56	360.00	155.56
Residue (R)	**SEM (±)**	**26.45**	**15.07**	**18.13**	**24.83**	**16.29**	**16.24**	**16.92**	**26.56**
	LSD (*P≤0.05*)	**80.24**	**45.72**	**55.00**	**NS**	**49.40**	**49.27**	**51.33**	**NS**
	B_0	493.33	373.33	262.22	146.67	546.67	440.00	315.56	186.67
	B_1	635.56	426.67	293.33	168.89	626.67	515.56	346.67	182.22
Bio fertilizers (B)	**SEM (±)**	**26.45**	**15.07**	**18.13**	**24.83**	**16.29**	**16.24**	**16.92**	**26.56**
	LSD (*P≤0.05*)	**80.24**	**45.72**	**NS**	**NS**	**49.40**	**49.27**	**NS**	**NS**
Interaction									
T X R		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
T X B		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
R X B		**NS**	**64.65**	**NS**	**NS**	**69.86**	**NS**	**NS**	**NS**
T X R X B		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Bivariate linear regressions help to understand and establishment between two variables. Here we want to find out the correlations between the SMBC and dehydrogenase activity in different depths under study irrespective of the year. The bivariate linear regression model (fig.3) revealed overall good regression coefficient (R^2) values were observed in all soil depths except in 20-40 cm soil depth. R^2 values of 0.74, 0.65 and 0.67 in 0-5 cm, 5-10 cm and 10-20 cm respectively indicating that there is a positive relationship between SMBC and DHA. Dehydrogenases have a strong correlation with microbial biomass (MB), hence influencing the breakdown of organic matter (OM) (Zhang et al., 2010). The relationship between soil enzymatic activity and soil organic matter level was apparent. An elevated organic matter level has the potential to supply a sufficient amount of substrate, hence facilitating an increase in microbial biomass and subsequent enzyme synthesis. A substantial positive association (r^2 = 0.71) between soil respiration and dehydrogenase enzyme indicated that this intracellular enzyme is involved in microbial respiration. Since dehydrogenase enzyme is only produced in living cells, dehydrogenase activity can indicate microbial activity and the whole range of soil microflora's oxidative activity (Bolton et al., 1985).

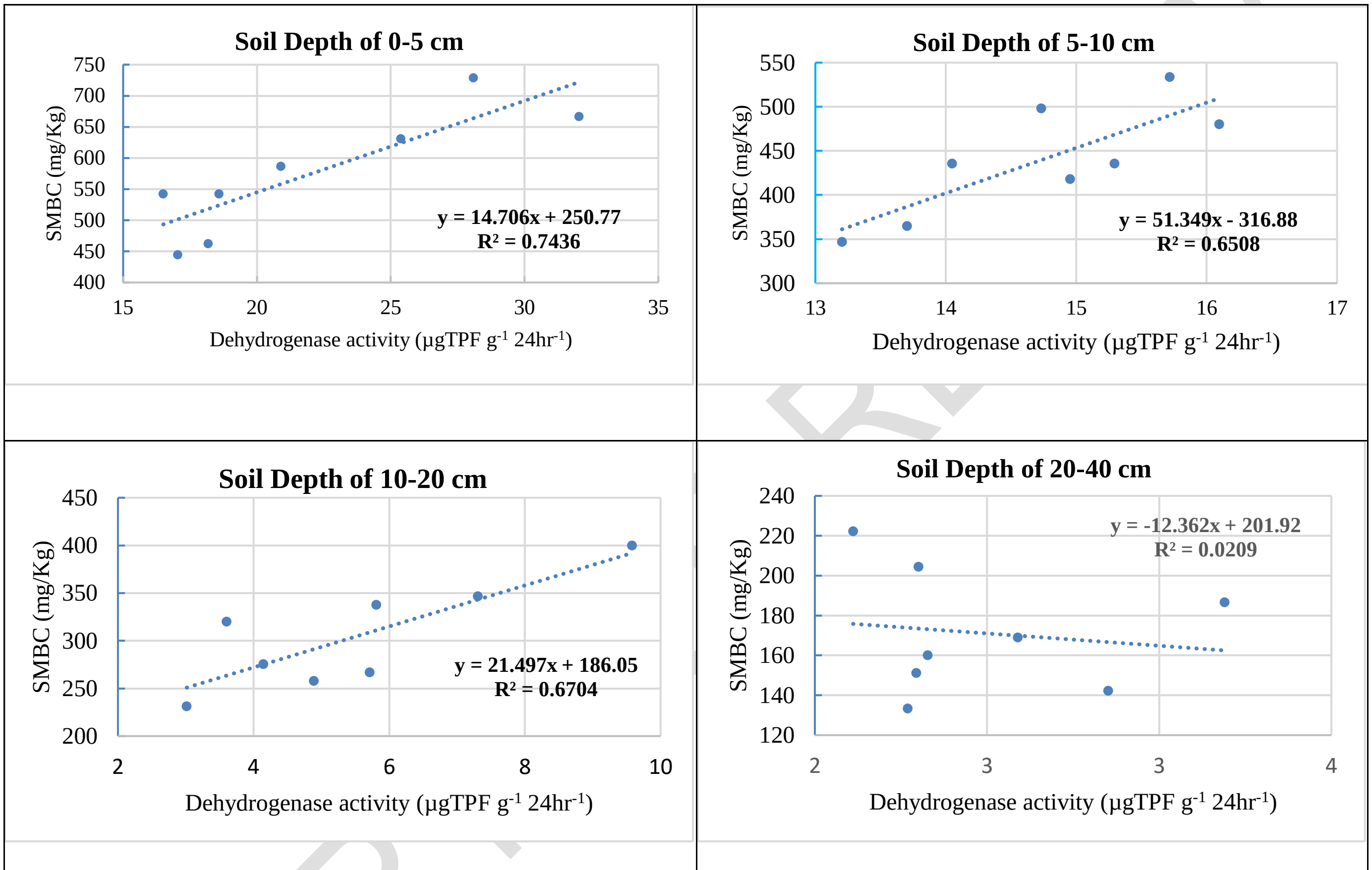


Fig. 3: Bivariate linear regressions between SMBC and Dehydrogenase activity

4. CONCLUSION

Long-term field experiment helps to crop production management practices affect soil chemical and biological system**.** Conservation tillage has been found to have an important positive impact on the rhizosphere soil environment, because of the intimate association between microorganisms in the rhizosphere soil and soil ecology. However, it is necessary to elucidate the regulatory mechanisms of no or reduced tillage on microbial diversity in the rhizosphere soil, taking into account variations in temperature, soil type, and management practices. Our results suggest that tillage, residue management, biofertilizer addition, cropping system, and their interactions have definite influence on dehydrogenase activity and SMBC. The findings of this study indicate that the presence of crop residue has a positive impact on microbial biomass and enzymatic activity. Additionally, the consistent and even supply of carbon through crop residues acts as a source of energy for microorganisms. When residue was retained, zero tillage catalyzes comparable or greater microbial biomass and micro-flora activity in comparison to conventional tillage, resulting to higher enzyme activity in soil.

Long-term field study conducted to investigate the effects of different management practices on soil biology, reveals that dehydrogenase activity, as a key indicator of soil health, was influenced by cropping systems, nutrient management practices, and the biofertilizers. The results show that dehydrogenase activity tends to decrease with soil depth, which can be attributed to differences in organic matter content, O_2 supply, and microbial activity. Notably, zero tillage (ZT) practices, which help maintaining crop residues and organic matter on the soil surface consistently resulted in higher dehydrogenase activity compared to conventional tillage (CT). The addition of crop residues and biofertilizers showed supplementary effect on dehydrogenase activity, especially in the top soil layers. The findings highlight the importance of organic matter in providing substrates for dehydrogenase enzymes and, consequently, in enhancing soil microbial activity. Furthermore, the study showed a strong positive correlation between soil microbial biomass carbon (SMBC) and dehydrogenase activity, underscoring the interplay between microbial activity and enzyme synthesis up to 20 cm of soil depth. This relationship emphasizes the role of organic matter in supporting both microbial growth and enzyme production. In summary, the research suggests that crop production practices, such as zero tillage and incorporation of crop residue and biofertilizer, can contribute to improve soil health by enhancing enzyme activity and microbial biomass with the implications of maintaining long-term soil productivity and environmental sustainability in agricultural systems.

COMPETING INTERESTS: Authors have declared that no competing interests exist

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