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# 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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***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

As a major supply of staple foods, the rice-wheat cropping system is crucial to global food security. However, continuous cultivation of these crops has led to several soil-related environmental problems, including the gradual decline in soil health and quality. In this context, maintaining soil health necessitates the adoption of conservation agriculture. This study assessed the effect of tillage, residue and biofertilizer management practices on soil dehydrogenase activity (SDHA) and soil microbial biomass carbon (SMBC) of post wheat soils at various soil depth (0-5 cm, 5-10 cm, 10-20 cm and 20-40 cm). The research was conducted as part of a long-term field experiment initiated in 2006 in rice-wheat cropping system under a factorial randomized block design with three replications in the Terai agro-ecological regions of West Bengal. The results revealed that the SDHA and SMBC were increased by the influence of long-term zero tillage, bio fertilizer & residue addition at the most of the soils depths under study except at 20-40 cm. The highest short-term change of SDHA (213.19%) in compared to the initial soil status was observed in conventionally tilled soil with biofertilizer addition treatment combination. Since SDHA and SMBC are critical indicators of soil health and quality, this study also explored their relationship. Positive good regression coefficient of 0.74, 0.65 and 0.67 at 0-5 cm, 5-10 cm and 10-20 cm soil depth were observed respectively. These findings highlight the strong association between the two parameters. With growing future demand of foods, conservation agriculture must be practiced to ensure the long-term sustainability of soil productivity. The study provides evidence that implementing zero tillage along with residue and biofertilizer addition significantly improves these key soil health parameters.

*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, intensifying the problem. 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 provide 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 activity and soil microbial biomass carbon as influenced by the long-term tillage, residue and bio-fertilizer management.
- To evaluate the short-term changes of soil dehydrogenase activity and soil microbial biomass carbon in comparison with the initial soil status.

## 2. MATERIALS AND METHODS

**Site Description:** A long-term field 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 shown in Table 1. We studied soil dehydrogenase activity (SDHA) and soil microbial biomass carbon (SMBC) at various soil depths after two consecutive rice-wheat crop cycles of the years 2020 and 2021 as 1st and 2nd crop cycles respectively. The results presented in this topic reflect the cumulative effect of treatment variables on SDHA and SMBC after 14-15 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 |
|---|---|
| $T_1$ ($ZTR_0B_1$) | Zero tillage (ZT) + Residue removed ($R_0$) + Bio-fertilizer ($B_1$) |
| $T_2$ ($ZTR_0B_0$) | Zero tillage (ZT) + Residue removed ($R_0$) + No bio-fertilizer($B_0$) |
| $T_3$ ($ZTR_1B_1$) | Zero tillage (ZT) + Residue added ($R_1$) + Bio-fertilizer($B_1$) |
| $T_4$ ($ZTR_1B_0$) | Zero tillage (ZT) + Residue added ($R_1$) + No bio-fertilizer ($B_0$) |
| $T_5$ ($CTR_0B_1$) | Conventional tillage (CT) + Residue removed($R_0$) + No bio-fertilizer($B_0$) |
| $T_6$ ($CTR_0B_0$) | Conventional tillage (CT) + Residue removed($R_0$) + No bio fertilizer($B_0$) |
| $T_7$ ($CTR_1B_1$) | Conventional tillage (CT) + Residue added ($R_0$) + Bio-fertilizer ($B_1$) |
| $T_8$ ($CTR_1B_0$) | Conventional tillage (CT) + Residue added ($R_0$) + No bio fertilizer($B_1$) |

**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 cm, 5-10 cm, 10-20 cm and 20-40 cm) after each rice-wheat crop cycle. The soil samples were brought to the laboratory in icebox to minimize the microbial activity and kept in the refrigerator in $4^0$C until analyzed.

### Soil Analysis:

**Dehydrogenase activity**: For determining dehydrogenase activity, 1 g 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).

**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

**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 (SDHA) (Wolinska and Stepniewska, 2012). Table 2 highlights the soil dehydrogenase activity across treatment combinations, showing a consistent decline with increasing soil depth in both the rice-wheat crop cycle. Soil dehydrogenase activity was noted a decrease with soil depth irrespective of the treatments in both years of study soils. After 1st rice wheat crop cycle, highest value of 29.27 µgTPF $g^{-1}$ $24hr^{-1}$ was observed in bioinnoculated zero tillage plot without residue added ($T_1$ - $ZTR_0B_1$) soils that was statistically ($p \leq 0.05$) at par with zero tillage with residue & bio-inoculants added soils ($T_3$- $ZTR_1B_1$) and zero tillage with residue addition ($T_4$- $ZTR_1B_0$) at 0-5 cm soil depth. At 5-10 cm soil layer, increased SDHA was noticed in $T_1$, which was statically at par with all the treatments except $T_2$ (zero tillage without bio-inoculants and residue), $T_4$ (zero tillage with residue addition only) and $T_6$ (CT without residue and bio-inoculants). Maximum SDHA was found in $T_7$ at both 10-20 cm & 20-40 cm soil layers, which being 10.71 and 2.93 µgTPF $g^{-1}$ $24hr^{-1}$ respectively.

After 2nd rice-wheat crop cycle, dehydrogenase activity recorded into the highest value (34.80 µgTPF $g^{-1}$ $24hr^{-1}$) at 0-5 cm soil depth. However, at 5-10 cm soil depth, maximum recorded into highest value of 16.93 µg TPF $g^{-1}$ $24hr^{-1}$ was recorded in $T_1$ treatment which was significantly higher than other treatments, except $T_2$, $T_5$ and $T_6$ which 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 conventionally tilled treatment with biofertilizer and residue addition ($T_7$). Significantly, highest values of 3.44 µg TPF $g^{-1}$ $24hr^{-1}$ was noticed in $T_2$ at 20-40 cm soil depth. Relatively higher values of dehydrogenase activity at surface layers (0-5 cm), 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. Effect of different treatment combinations on soil dehydrogenase activity (µg TPF g-1 24hr-1) at different soil depths**

| Treatment | After 1st rice-wheat crop cycle | | | | After 2nd rice-wheat crop cycle | | | |
|---|---|---|---|---|---|---|---|---|
| | 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 |

**Table 3. Main effect and interaction effect of tillage, residue and biofertilizer management practices on soil dehydrogenase activity (µg TPF g-1 24hr-1) in soils at different depths after 1st and 2nd rice-wheat crop cycle**

| **Treatment** | | **After 1st rice-wheat crop cycle** | | | | **After 2nd rice-wheat crop cycle** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **0-5 cm** | **5-10 cm** | **10-20 cm** | **20-40 cm** | **0-5 cm** | **5-10 cm** | **10-20 cm** | **20-40 cm** |
| Tillage (T) | 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 |
| | 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 |
| Residue (R) | $R_0$ | 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 |
| | 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 |
| Bio fertilizers (B) | $B_0$ | 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 |
| | 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 |

It is evidenced from the results (Table 3) that in both the rice-wheat crop cycles SDHA in zero tillage was significantly ($p \le 0.05$) higher than the conventional tillage system in all the soil depths under study except in 5-10 cm layer in 1st crop cycle. Residue addition significantly increased that SDHA at 5-10 cm soil depth in 2nd crop cycle and at 10- 20 cm soil depth in both rice-wheat crop cycle. Biofertilizers inoculation proved to increase significantly ($p \le 0.05$) this enzymatic activity in all the soil depths except 10- 20 cm soil depth in 1st crop cycle and 20-40 cm in both crop cycles. Interaction effect of tillage, residue and biofertilizers are found significant in 0-5 cm soil depth in both crop cycles and 10- 20 cm soil depth in 1st crop cycle. 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 favor 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 residue management strategies. Similar increases in enzyme activity were seen in plots where crop 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 also. Manjaiah and Singh, 2001 found in their study that increased availability of nutrients for microbial metabolism may account for the beneficial effect of crop residue and Leucaena mulch on dehydrogenase activity in addition to providing nutrients, crop leftovers may also affect the soil's moisture, temperature, and aeration. 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.

Short term change (%) of soil DHA compared to the start of the experiment of different treatment combinations at different soil depths are depicted in Fig.1. In this figure, it is evidenced that there was increment of SDHA at all the soil depths and treatments except 10-20 cm depth of $T_2$ ($ZTR_0B_0$). Maximum positive change of Soil DHA of 213.19 % was noticed at 10-20 cm soil depth of $T_5$ which was conventionally cultivated with bio fertilizer inoculation than the other treatment combinations and soil depth.

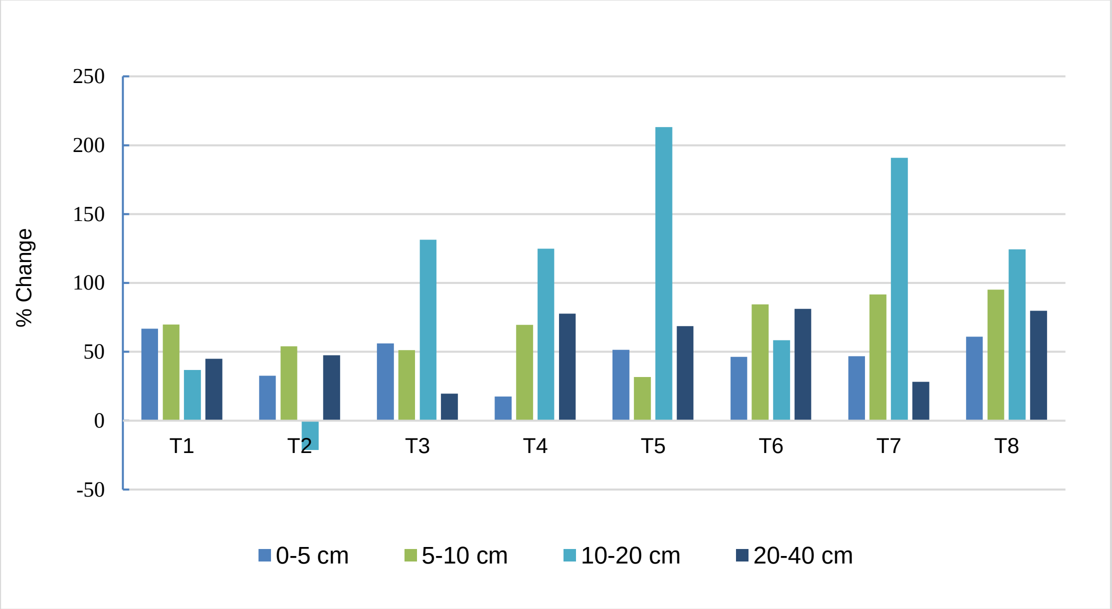

**Fig. 1. Short term changes of Soil DHA**

**Effect of Tillage Residue and Bio Fertilizer Management Practices on soil Microbial Biomass Carbon (SMBC) after 1st and 2nd Rice-Wheat Crop Cycle:** Soil microbial biomass carbon as influenced by the long-term tillage, residue and biofertilizer management after two consecutive rice-wheat crop cycle have shown in Table 4. The microbial biomass is considered to be very susceptible to change and is a significant component of the organic matter pool. An increase of 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 SMBC values as stated in Table 4 ranges from 124.44 to 764.44 mg C Kg$^{-1}$ across various soil depths irrespective of crop cycle. After 1st rice-wheat crop cycle, at 0-5 cm soil, highest value of 764.44 mg/Kg was noted in $T_3$ ($ZTR_1B_1$), which was statistically at par with $T_1$ ($ZTR_0B_1$) & $T_4$ ($ZTR_1B_0$), and lowest value of 426.67 mg/Kg was observed in conventionally tilled without biofertilizer and residue treatment plots ($T_6$). A notable increase of SMBC was observed in $T_3$ and $T_4$ where residue addition with zero tillage was practiced. The presence of easily metabolizable and hydrolyzable carbon and nitrogen in crop residues, as well as reduced soil disturbance due to zero tillage may be the reason of increased MBC in $T_3$ ($ZTR_1B_1$) treatment plot than the other treatment combinations. 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 2nd rice wheat crop cycle, at 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 $T_1$($ZTR_0B_1$), $T_4$($ZTR_1B_0$), $T_7$ ($CTR_1B_1$) and $T_8$ ($CTR_1B_0$). 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 $T_5$. At 10-20 cm and 20-40 cm soil layers, no significant difference among the treatments was observed in both the crop cycles of study.

Main effect of tillage, residue and biofertilizer management practices on SMBC at different soil depths of two consecutive rice-wheat crop cycle have shown in Table 5. Zero tillage (ZT) have proved to significantly ($p \le 0.05$) increase Soil MBC over conventional tilled (CT) in 0-5 cm and 5-10 cm soil depth in both the rice-wheat crop cycle. But at 10- 20 cm soil depth SMBC was recorded higher in CT than ZT in both the crop cycles. Residue addition ($R_1$) significantly showed higher SMBC at all the soil depths except 20-40 cm in both crop cycles than the residue removed soils ($R_0$). Effect of biofertilizer addition on SMBC was confined up to the 10 cm soil depth only. Biofertilizer inoculation ($B_1$) significantly increase the soil MBC at 0-5 cm and 5-10 cm soil depth than the no bio-inoculated soils ($B_0$) in both the crop cycles.

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. The addition of crop residue with biofertilizer have 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 residue 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. Crop residues provide a consistent and homogeneous supply of carbon, as an energy source for microorganisms. The implementation of zero tillage with residue removal led to a decrease in soil microbial biomass (SMB), as seen in Table 4. Conversely, zero tillage (ZT) with residue retention demonstrated the ability to sustain a high level of SMBC. 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).

**Table 4. Effect of different treatment combinations on microbial biomass carbon (mg/Kg) at different soil depths**

| | After 1st rice-wheat crop cycle | | | | After 2nd rice-wheat crop cycle | | | |
|---|---|---|---|---|---|---|---|---|
| **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 ($P \leq 0.05$) | 160.48 | 91.43 | NS | NS | 98.80 | 98.54 | NS | NS |

Short term change (%) of soil MBC compared to the start of the experiment of different treatment combinations at diffrent soil depths 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 $T_2$ (ZTR$_0$B$_0$), 20-40 cm in $T_4$(ZTR$_1$B$_0$), 5-10 cm in $T_6$ (CTR$_0$B$_0$) and 5-10 cm in $T_8$(CTR$_1$B$_0$). 180.00 % increment of SMBC was noticed in $T_2$ (ZT with residue removed and no bio inoculation plot) of 5-10 cm soil depth. At 5- 10 cm of $T_8$ (CT with residue addition and no bio inoculated treatment plot) 16.67 % reduction of SMBC was noticed.

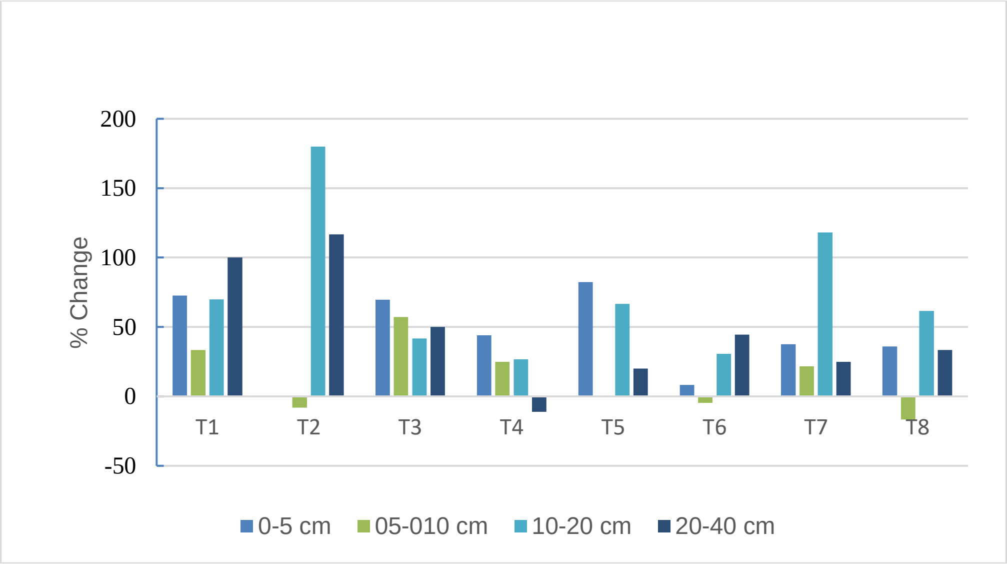

**Fig. 2. Short term changes of SMBC**

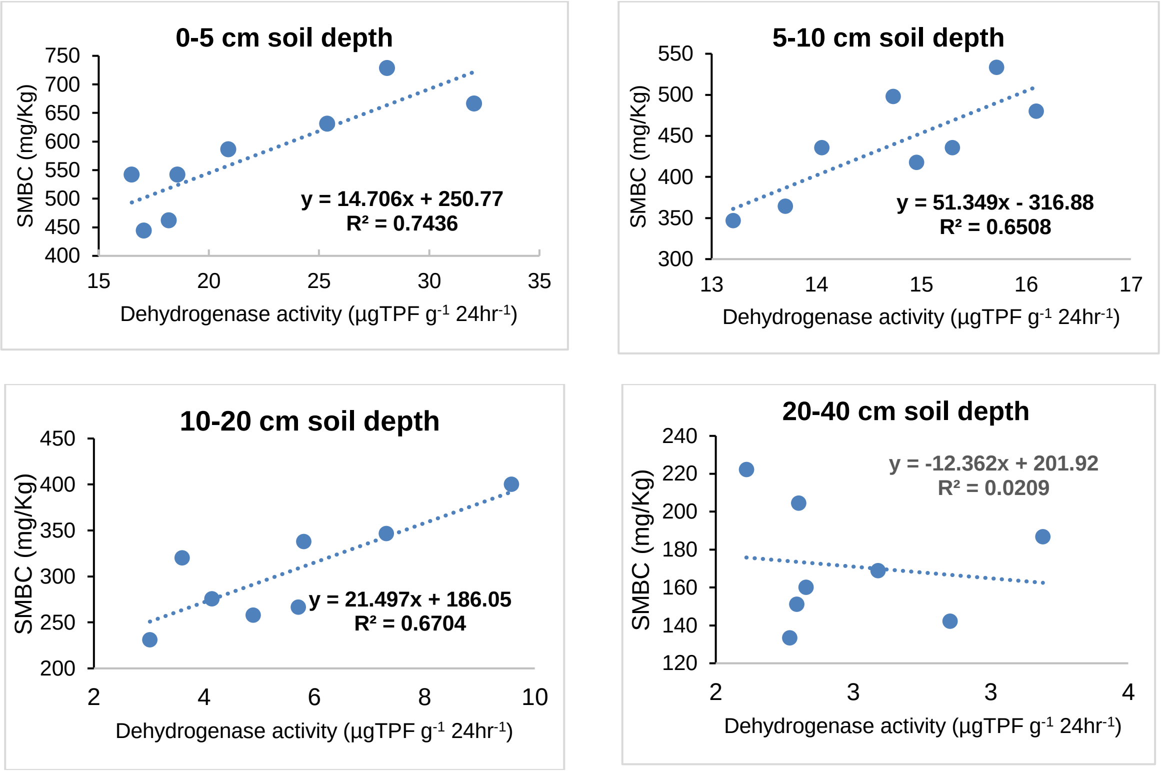

**Fig. 3. Bivariate linear regressions between SMBC and Dehydrogenase activity**

**Table 5. Main and interaction effect of tillage, residue and biofertilizer management practices on soil microbial biomass carbon (mg/Kg) in soils at different depths after 1st and 2nd rice-wheat crop cycle**

| **Treatment** | | **After 1st rice-wheat crop cycle** | | | | **After 2nd rice-wheat crop cycle** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **0-5 cm** | **5-10 cm** | **10-20 cm** | **20-40 cm** | **0-5 cm** | **5-10 cm** | **10-20 cm** | **20-40 cm** |
| Tillage (T) | 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 |
| | SEM (±) | 26.45 | 15.07 | 18.13 | 24.83 | 16.29 | 16.24 | 16.92 | 26.56 |
| | LSD ($P \leq 0.05$) | 80.24 | 45.72 | 55.00 | NS | 49.40 | 49.27 | 51.33 | NS |
| Residue (R) | $R_0$ | 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 |
| | SEM (±) | 26.45 | 15.07 | 18.13 | 24.83 | 16.29 | 16.24 | 16.92 | 26.56 |
| | LSD ($P \leq 0.05$) | 80.24 | 45.72 | 55.00 | NS | 49.40 | 49.27 | 51.33 | NS |
| Bio fertilizers (B) | $B_0$ | 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 |
| | SEM (±) | 26.45 | 15.07 | 18.13 | 24.83 | 16.29 | 16.24 | 16.92 | 26.56 |
| | LSD ($P \leq 0.05$) | 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 find out the correlations between the SMBC and dehydrogenase activity in different depths under study irrespective of the 1st and 2nd crop cycle. The bivariate linear regression model (Fig.3) revealed overall good regression coefficient ($R^2$) values at 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 SDHA. Zhang et al., 2010 reported that dehydrogenase activity have a strong correlation with microbial biomass (MB), hence influencing the breakdown of soil organic matter. The relationship between soil enzymatic activity and soil organic matter level was apparent. Increased organic matter level has the potential to supply a sufficient amount of substrate, hence facilitating an increase in microbial biomass and subsequent dehydrogenase 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, Monokrousos et al. 2008, Franzluebbers et al. 1999).

## 4. CONCLUSION

Long-term field experiment offers opportunities to investigate the effect on different soil chemical and biological properties**.** 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.

The results have shown 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 to maintain 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 and dehydrogenase activity, underscoring the interplay between microbial activity and enzyme synthesis up to 20 cm of soil depth. 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.

## 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

## REFERENCES

Ahmad, I., & Iram, S. (2006). Rice–wheat cropping pattern and resource conservation technologies. *Agriculture Overview, Pakistan.com*. Available at: http://www.pakissan.com/english/agri.overview/rice.wheat.cropping.pattern.html (Accessed March 21, 2021).

Banerjee, B., Aggarwal, P. K., Pathak, H., Singh, A. K., & Chaudhary, A. (2006). Dynamics of organic carbon and microbial biomass in alluvial soil with tillage and amendments in rice-wheat systems. *Environmental Monitoring and Assessment, 119*, 173-189.

Banjara, T. R., Bohra, J. S., Kumar, S., Ram, A., & Pal, V. (2021). Diversification of rice–wheat cropping system improves growth, productivity and energetics of rice in the Indo-Gangetic region.

Bolton, H., Elliot, L. F., Papendick, R. I., & Benzdick, D. F. (1985). Soil microbial biomass and selected soil enzyme activities: Effect of fertilization and cropping practices. *Soil Biology & Biochemistry, 17*, 297-302.

Bowles, T. M., Acosta-Martínez, V., Calderón, F., & Jackson, L. E. (2014). Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. *Soil Biology and Biochemistry, 68*, 252-262.

Burns, R. G., & Dick, R. P. (Eds.). (2002). *Enzymes in the environment: Activity, ecology, and applications*. Marcel Dekker Inc.

Carter, M. R. (1992). Influence of reduced tillage systems on organic-matter, microbial biomass, macro-aggregate distribution, and structural stability of the surface soil in a humid climate. *Soil & Tillage Research, 23*, 361–372.

Casida Jr, L. E., Klein, D. A., & Santoro, T. (1964). Soil dehydrogenase activity. *Soil Science, 98*(6), 371-376.

Dhanda, S., Yadav, A., Yadav, D. B., & Chauhan, B. S. (2022). Emerging issues and potential opportunities in the rice–wheat cropping system of North-Western India. *Frontiers in Plant Science, 13*, 832683.

Franzluebbers, A. J., Nazih, N., Stuedemann, J. A., Fuhrmann, J. J., Schomberg, H. H., & Hartel, P. G. (1999). Soil carbon and nitrogen pools under low- and high-endophyte infected tall fescue. *Soil Science Society of America Journal, 63*, 1687–1694.

Jat, M. L., Jat, H., Agarwal, T., Bijarniya, D., Kakraliya, S. K., Choudhary, K. M., et al. (2020). A compendium of key climate-smart agriculture practices in intensive cereal-based systems of South Asia. *International Maize and Wheat Improvement Center (CIMMYT)*, New Delhi, India, 42.

Jenkinson, D. S. (1988). Determination of microbial biomass carbon and nitrogen in soil. In J. R. Wilson (Ed.), *Advances in Nitrogen Cycling in Agricultural Ecosystems* (pp. 368–386). Wallingford: CAB International.

Kumar, A., Mishra, V. N., Biswas, A. K., & Somasundaram, J. (2018). Soil organic carbon, dehydrogenase activity and fluorescein diacetate as influenced by contrasting tillage and cropping systems in Vertisols of Central India. *Journal of Environmental Biology, 39*(6), 1047-1053.

Lalik, R., Sharma, S., Idris, M., Singh, A. K., Singh, S. S., Bhatt, B. P., et al. (2014). Integration of conservation agriculture with best management practices for improving system performance of the rice–wheat rotation in the eastern Indo-Gangetic Plains of India. *Agriculture, Ecosystems & Environment, 195*, 68–82. https://doi.org/10.1016/j.agee.2014.06.001

Mahmud, A. A., Upadhyay, S. K., Srivastava, A. K., & Bhojiya, A. A. (2021). Biofertilizers: A nexus between soil fertility and crop productivity under abiotic stress. *Current Research in Environmental Sustainability, 3*, 100063.

Manjaiah, K. M., & Singh, D. (2001). Soil organic matter and biological properties after 26 years of maize-wheat-cowpea cropping as affected by manure and fertilization in a cambisol in semiarid region of India. *Agriculture, Ecosystems & Environment, 86*, 155–162.

Mijangos, I., Pérez, R., Albizu, I., & Garbisu, C. (2006). Effects of fertilization and tillage on soil biological parameters. *Enzyme and Microbial Technology, 40*(1), 100-106.

Monokrousos, N., Papatheodorou, E. M., & Stamou, G. P. (2008). The response of soil biochemical variables to organic and conventional cultivation of *Asparagus sp. Soil Biology and Biochemistry, 40*, 198–206.

Sahoo, S., Mukhopadhyay, P., Mowrer, J., Maity, P. P., Maity, A., Sinha, A.K., Sow, P., & Rakesh, S. (2022). Tillage and N-source affect soil fertility, enzymatic activity, and crop yield in a maize–rice rotation system in the Indian Terai zone. *Frontiers in Environmental Science, 10*, 983973.

Salinas-Garcia, J. R., Baez-Gonzalez, A. D., Tiscareno-Lopez, M., & Rosales-Robles,

E. (2001). Residue removal and tillage interaction effects on soil properties under rain-fed corn production in Central Mexico. *Soil and Tillage Research, 59*(1-2), 67-79.

Sharma, P. K., & Bhushan, L. (2001). Physical characterization of a soil amended with organic residues in a rice–wheat cropping system using a single value soil physical index. *Soil and Tillage Research, 60*, 143–152.

Singh, G., Bhattacharyya, R., Das, T. K., Sharma, A. R., Ghosh, A., Das, S., & Jha, P. (2018). Crop rotation and residue management effects on soil enzyme activities, glomalin and aggregate stability under zero tillage in the Indo-Gangetic Plains. *Soil and Tillage Research, 184*, 291-300.

Singh, Y., Singh, B., Meelu, O. P., & Khind, C. S. (2000). Long term effects of organic manuring and crop residues on the productivity and sustainability of rice–wheat cropping system of North-West India. In I. P. Abrol, K. F. Bronson, J. M. Duxbury, & R. K. Gupta (Eds.), *Long-term soil fertility experiments in rice-wheat cropping systems* (RWC Paper series 6, pp. 149–162). New Delhi: RWCIGP.

Sinsabaugh, R. L., & Follstad Shah, J. J. (2011). Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. *Biogeochemistry, 102*, 31-43.

Watts, D. B., Torbert, H. A., Feng, Y., & Prior, S. A. (2010). Soil microbial community dynamics as influenced by composted dairy manure, soil properties, and landscape position. *Soil Science, 175*, 474–486.

Wolinska, A., & Stepniewska, Z. (2012). Dehydrogenase activity in the soil environment. In R. A. Canuto (Ed.), *Dehydrogenases* (pp. 1-16). Intech, Rijeka. Available at: http://www.ebook3000.com/

Zhang, Y. L., Chen, L. J., Chen, Z. H., Sun, L. X., Wu, Z. J., & Tang, X. H. (2010). Soil nutrient contents and enzymatic characteristics as affected by 7-year no-tillage under maize cropping in a meadow brown soil. *Revista de la Ciencia del Suelo y Nutrición Vegetal, 10*, 150–157.

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