

# LONG-TERM IMPACTS OF ORGANIC INPUTS AND TILLAGE PRACTICES ON SOIL HEALTH, SOC SEQUESTRATION, AND CROP YIELD UNDER MAIZE CROP

**AYESHA FARZAND \***

Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.  
\*Corresponding Author Email: ayeshasara300@gmail.com

**MUHAMMAD SANA ULLAH**

Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.

**ABDUL WAKEEL**

Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan.

**IMRAN KHAN**

Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.

## Abstract

Agricultural practices like tillage, residues burning, incorporation, mulching and removal of crop residues influence soil health and its physical properties. These agricultural practices also contribute almost 20% of the global greenhouse gases (GHGs) emission which further contribute towards global warming (GW). The main purpose of the study was to elucidate the effects of wheat residue, its biochar and their application methods (incorporation vs mulching) on soil organic carbon (SOC) dynamics in maize crop. The study is helpful to the farming community in choosing better form of organic amendment which contributes less in GHGs emissions and climate change. Therefore, a field trial was conducted by using maize (*Zea Mays L.*) as test crop. Wheat residue ( $2 \text{ t ha}^{-1}$ ) and its biochar was incorporated in soil and mulched on soil. A control without any organic amendment was also maintained. Each treatment had four replicates distributed according to randomized complete block design (RCBD). Soil carbon dioxide emissions are measured by trapping the  $\text{CO}_2$  in gas chamber using  $1 \text{ M NaOH}$  and  $\text{NH}_3$  by trapping in  $2\% \text{ H}_3\text{BO}_3$  once in a week. At the end of experiment SOC, microbial biomass carbon (MBC), extracellular enzymes of soil and soil aggregate fractionation were determined. The results showed that  $\text{CO}_2$  emissions were significantly increased by application of wheat residues ( $2634 \pm 179 \text{ mg C m}^{-2} \text{ day}^{-1}$ ) and wheat biochar ( $2325 \pm 86 \text{ mg C m}^{-2} \text{ day}^{-1}$ ) as incorporation as compared to mulching. Volatilization of  $\text{NH}_3$  was higher in application of wheat residues as incorporation ( $690 \pm 20 \text{ mg NH}_3 \text{ m}^{-2} \text{ day}^{-1}$ ) and wheat biochar ( $633 \pm 57 \text{ mg NH}_3 \text{ m}^{-2} \text{ day}^{-1}$ ) at 45<sup>th</sup> day and then it decreased till 75<sup>th</sup> day. Soil MBC was significantly increased by application of wheat residue as mulching as compared to other. The  $\beta$ -glucosidase enzyme activity was significantly increased by application of wheat biochar as incorporation ( $88 \pm 17 \text{ n M g}^{-1} \text{ soil hr}^{-1}$ ) and significant increase in chitinase activity was observed by application of wheat residue and wheat biochar as mulching as compared to incorporation. Acid phosphatase activity showed highly significant increase in application of wheat biochar as incorporation ( $413 \pm 82 \text{ n M g}^{-1} \text{ soil hr}^{-1}$ ). Total grain weight yield was significantly increased in incorporation of wheat residue and wheat biochar as compared to mulching and no amendment. SOC was significantly increased by application of wheat residue as incorporation and wheat biochar as mulching as compared to no amendment. It was concluded that yield and  $\beta$ -glucosidase enzyme activity increased by addition of wheat biochar. Microbial biomass carbon, soil organic carbon and soil nitrate were increased by

addition of wheat residue as incorporation. Hence, it is suggested to use wheat residue and its biochar as organic amendments to improve soil organic carbon sequestration and soil physical characteristics.

## 1. INTRODUCTION

Sustainable agriculture relies heavily on effective soil management practices that enhance soil health, improve productivity, and contribute to environmental conservation (Tahat et al., 2020; Martínez-Mena et al., 2020). Among these practices, the use of organic inputs (such as manure, compost, and crop residues) and tillage methods (ranging from conventional tillage to conservation practices like reduced tillage or no-till) are key strategies for maintaining soil fertility, enhancing soil organic carbon (SOC) sequestration, and sustaining crop yields (Lal et al., 2020; Telles et al., 2022). However, understanding the long-term effects of these practices remains crucial to optimizing their benefits across different agro-ecological conditions.

Organic inputs play a crucial role in enhancing soil conditions by improving structure, porosity, water-holding capacity, and nutrient availability, which are vital for maintaining soil-plant-environment interactions and building resilience against the impacts of climate change. These materials, when applied consistently over time, contribute to the accumulation of organic matter, which enhances microbial activity, promotes soil aggregation, and improves root development and water infiltration (Farzand et al., 2023; Mandal et al., 2021; Hou et al., 2020). Additionally, organic amendments play a vigorous role in increasing SOC, a critical component of soil health that helps retain nutrients and water, supports soil biological activity, and contributes to mitigating climate change by sequestering the atmospheric carbon dioxide (CO<sub>2</sub>) (Nazir et al., 2024; Veni et al., 2020; Rastogi et al., 2023).

Tillage practices also significantly affect soil properties and carbon dynamics. Conventional tillage, involving intensive plowing and soil turnover, often leads to soil structure disruption, increased organic matter decomposition, and heightened soil erosion (Alvarenga et al., 2020; Rahman et al., 2020). In contrast, conservation tillage methods, such as reduced tillage or no-till, aim to minimize soil disturbance, retain crop residues on the surface, and maintain organic matter levels. These practices have been shown to enhance SOC sequestration, improve soil moisture retention, and reduce erosion (ur Rehman et al., 2023; Hussain et al., 2021). However, the extent of these benefits can vary depending on soil type, climate, and management practices.

SOC sequestration is a crucial process for improving soil health and mitigating climate change. SOC is a key indicator of soil quality, influencing its capacity to retain water and nutrients, support microbial activity, and sustain plant growth (Rodrigues et al., 2023; Elbasiouny et al., 2022; Das et al., 2021). Sustainable soil management practices that combine organic inputs with conservation tillage have the potential to increase SOC levels by balancing carbon inputs from plant residues and organic amendments with losses due to decomposition and erosion. Understanding the factors that affect SOC dynamics is essential for developing effective land management strategies that support both

agricultural productivity and environmental sustainability (Littrell et al., 2021; Sarkar et al., 2020).

Despite the recognized benefits of organic inputs and conservation tillage, there is limited research on their combined long-term effects on soil properties, SOC sequestration, and crop yields, especially under varying environmental conditions. Most existing studies focus on short-term outcomes or specific contexts, overlooking the cumulative effects of these practices over multiple growing seasons. This gap is particularly evident in semi-arid regions, where maintaining soil fertility and water-use efficiency are critical challenges for sustainable agriculture.

In the context of maize production, understanding the long-term impacts of organic inputs and tillage practices is essential for developing sustainable farming strategies. Maize, a staple crop globally, is highly dependent on soil health and fertility for optimal growth and yield (Akram et al., 2023; Steponavičienė et al., 2024). However, there is a gap in knowledge regarding the combined effects of organic inputs and different tillage practices on soil health, SOC sequestration, and maize crop yield over the long term. While some studies have explored the individual impacts of these practices, there is limited research on their combined effects and how these interactions influence the overall sustainability of maize production systems (Maenhout et al., 2024; Pearsons et al., 2023).

This research aims to evaluate the long-term effects of organic inputs and tillage practices on soil health, SOC sequestration, and maize crop yield. By assessing the impacts of these practices over an extended period, this study seeks to provide insights into the most effective management strategies for enhancing soil quality, maximizing SOC sequestration, and achieving sustainable crop production. The findings will contribute to a better understanding of sustainable agricultural practices and their role in addressing the challenges of food security, climate change, and soil degradation.

## 2. MATERIALS AND METHODS

Field experimentation was conducted at farm of the Institute of Soil and Environmental Sciences (31.4395 °N, 73.0704 °E), University of Agriculture Faisalabad, Pakistan for maize crop during the growing season of 2023. Land was prepared by tillage implements in which two tillage depths were maintained. Cultivator was used for conventional tillage (0-15 cm) and Chisel plow was used for deep tillage (0-30 cm). Wheat residue was collected from farm area which was further chopped, dried and applied as incorporation and mulch uniformly in respective treatment plots of 30 m<sup>2</sup> at the rate of 2 ton ha<sup>-1</sup>. Maize (variety Faisalabad-2008) was sown as test crop at recommended rate of 123 kg ha<sup>-1</sup>. Recommended dose of fertilizers N, P, K (133:83:61) kg ha<sup>-1</sup> respectively was applied in form of urea, di-ammonium phosphate and sulfate of potash. Half dose of N and full dose of P and K were broadcast at the time of sowing, while another dose of N was broadcast with second irrigation.

## 2.1 Experimental design

The experiment consisted of six treatments having conventional tillage (CT, 15 cm) with wheat residue and its biochar application as incorporation and control having no residue and zero tillage with wheat residue and its biochar application as mulching and control having no residue. Each treatment had four replications distributed according to RCBD.

## 2.2 Gaseous emissions

### a. Carbon dioxide efflux

Carbon dioxide trapped in 1 M NaOH solution was measured by back titration with 0.1 M HCl solution. 20 mL of NaOH trap solution was used for trapping after that 1 mL of trapped solution, 6-7 drops of BaCl<sub>2</sub> solution was added in beaker. Then pinkish hue appeared after adding phenolphthalein indicator (2-3 drops), after this it was titrated gradually until colorless termination point observed. Three blanks of NaOH were titrated as well (Anderson, 1983; Kuzyakov and Cheng, 2001).

$$\text{Total CO}_2 \text{ conc.} = \frac{(12 \times V1 \times 0.1)}{2 \times V2} \times (B - V3)$$

Here:

12: Atomic mass of C

V1: Volume of NaOH used for trapping of CO<sub>2</sub> (20 mL)

0.1: Molarity of HCl

2: Conversion factor

V2: Volume of NaOH used for titration (1 mL)

B: Blank average

V3: Volume of HCl used for titration

### b. Ammonia Volatilization

Ammonia gas that is trapped in 20 mL of 2% boric acid solution was calculated by back titration with 0.005 M H<sub>2</sub>SO<sub>4</sub>. 5 mL of trapped boric acid was taken in beaker then add 2-3 drops of methyl red indicator, greenish color appeared then slowly back titrated with 0.005 M H<sub>2</sub>SO<sub>4</sub> solution till red color endpoint reached (Bremner and Keeney, 1965).

$$\text{Total NH}_3 \text{ conc.} = \frac{(14 \times V1 \times 0.005 \times 20 \times 1000)}{0.328 - 0.02}$$

Here:

14: Atomic mass of N

V1: Volume of H<sub>2</sub>SO<sub>4</sub> used for trapping of NH<sub>3</sub>

0.005: Molarity of H<sub>2</sub>SO<sub>4</sub>

20: Volume of boric acid used for titration in mL

1000: Factor

0.328: Area of chamber

0.02: Boric acid concentration

## 2.3. Soil pre-analysis

### a. Sample collection and preparation

Soil samples were collected from the experimental area at two depths 0-15 cm and 15-30 cm. Roots were separated. Sample was dried and large aggregates were crushed into smaller aggregates. Sieve with 2 mm of size and made composite sample from each experimental plot which was further used for analysis of soil.

### b. Soil pH determination

The pH of the soil was checked from saturated paste of the soil by taking 250 g of the soil in the beaker and added deionized water until this paste attained standard conditions. When saturated paste was prepared standardize the pH meter with buffer solution of different levels i.e., 4, 7, and 10 pH and dipped pH meter probe in saturated paste in such a manner that it had not impacted the bottom and walls of beaker. Model of pH meter (HANNA Model HI9811-5) (U.S. Salinity Laboratory Staff, 1954; Method 21a).

### c. Electrical conductivity

Electrical conductivity of soil was determined from soil extract obtained from filtering saturated paste through Whatman No. 42 filter paper using vacuum pump. The extract was transferred into beaker and inserted EC meter in such a way that probe fully dipped in extract. The electrical conductivity meter was calibrated with 0.01 N KCl solution and noted the reading using a digital portable conductivity meter of given model (HANNA Model HI9811-5). The cell constant and the definite EC value was determined by

$$\text{Cell constant (K}_c\text{)} = \frac{1.4118 \text{ dS m}^{-1}}{\text{EC of 0.01 N KCL solution (dS m}^{-1}\text{)}}$$
$$\text{Actual EC}_e\text{(dS m}^{-1}\text{)} = \text{K}_c \times \text{Observed EC (dS m}^{-1}\text{)}$$

### d. Soil texture

Soil texture was checked through hydrometer technique recommended by (Bouyoucos, 1962). For this, 40 g of soil were placed in a 500 mL glass beaker, along with 60 mL of a dispersal solution that was composed by combining 40 g of the given chemical i.e., sodium hexametaphosphate and 10 g of the sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) in distilled water. The glass beaker was then left overnight with a watch glass on top. The solution was poured into a blending cup, swirled for about 3 minutes, and then placed in a 1 L graduated cylinder with distilled water until the mark was reached. When the homogeneous mixture was made using plunger, it took 1st reading of hydrometer after forty seconds and 2<sup>nd</sup> reading after almost two hours by putting hydrometer into suspension. Temperature of the mentioned instrument was retained at 20 °C. For temperature correction, add or subtract 0.4 factor by each degree increase or decrease from 20 °C respectively.

### e. Soil organic matter

Soil organic matter was found by a method that was introduced by (Walkley and Black, 1934). About 0.5 g quantity of soil sample was balanced and added 5 mL of the solution Potassium dichromate ( $K_2Cr_2O_7$ ) and 10 ml of 98% Sulphuric acid ( $H_2SO_4$ ) mixed well and stand for cooling up to 30 mints.

After cooling 100 mL of the DI water was added then 10 mL of the concentrated phosphoric acid ( $H_3PO_4$ ). Then, 10-12 drops of indicator diphenylamine were added, and then the prepared solution was titrated using 0.5 M solution of the ferrous ammonium sulfate until an unsightly green hue emerged.

Along with the samples three blank samples were also titrated.

Calculation of % O.M was done with the following formulas:

$$\% \text{ organic matter} = \frac{(V_1 - V_2) \times 0.69 \times M}{\text{Wt. of dry soil (g)}}$$

$V_1$  = Volume of ferrous ammonium sulfate used for blank

$V_2$  = Volume of ferrous ammonium sulfate used for sample

M = Molarity of ferrous ammonium sulfate

0.69 =  $0.003 \times 100 \times 100 / 72 \times 100 / 58$

100 = conversion factor of OM into %

100/58 = conversion factor of OC to OM

110/72 = carbon recovery factor

### f. Soil nutrient analysis

#### 1) Extractable phosphorus

The soil's native P content was assessed using the sodium bicarbonate ( $NaHCO_3$ ) technique. The pH of the sodium bicarbonate (0.5 N) solution was maintained at 8.5 by adding 5 N NaOH.

Ammonium Hepta-Molybdate (12 g) and Antimony Potassium Tartrate (0.2908 g) were mixed in 250 mL and 100 mL of distilled water respectively and poured into a 2 L volumetric flask and mixed well.

Then, 5 N  $H_2SO_4$  (1 L) was added into this volumetric flask and 2 L volume was made with distilled water. This mixture was named reagent A.

200 mL of reagent A were added with 1.05 g of L-ascorbic acid, and the mixture was thoroughly mixed.



This mixture was named reagent B. For preparation of standards,  $\text{KH}_2\text{PO}_4$  (0.2197 g) was dissolved in distilled water to make 1 L volume and standard stock solution (50 ppm) was prepared. From this standard stock solution, respective standards (0.5, 1, 1.5, 2.0, and 2.5 ppm) were made. For standardization of instrument, 2 mL of every normal solution was added into a 250 mL Erlenmeyer flask.

The mixture of soil (5 g) and 0.5 M  $\text{NaHCO}_3$  (10 mL) was agitated at 200–300 rpm for 30 minutes before being filtered through Whatman No. 40 filter paper. Clear filtrate of 10 mL, 1 mL of 5 N  $\text{H}_2\text{SO}_4$  (for pH adjustment to 5) and 8 mL of reagent B were added into a 50 mL flask.

A calibration curve was created once the instrument was regulated with a series of relevant standards. On a spectrophotometer, the sample's absorbance of light at 882 nm wavelength was measured (Thermo Electron). Plotting the sample's absorbance against the values of the relevant standards on a standard curve allowed researchers to determine the actual P concentration.

Extractable P ( $\text{mg kg}^{-1}$ ) was determined by using formula.

$$\text{Extractable P (mg kg}^{-1}\text{)} = \frac{\text{P (mg kg}^{-1}\text{) from standard curve} \times V \times V_2}{\text{Weight of dried soil (g)} \times V_1}$$

Where,

$V$  = Total volume of soil extract (mL)

$V_1$  = Volume of soil extract used for measurement (mL)

$V_2$  = Volume of flask used for measurement (mL)

## 2) Soluble potassium

Ammonium acetate (1 N) was prepared by dissolving the concentrated acetic acid ( $\text{CH}_3\text{COOH}$ ) and concentrated ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) in 1 L volumetric flask. For instrument calibration, stock solution of 1000 ppm K concentration was made by dissolving dried KCl (1.907 g) in 1000 mL of distilled water in 1 L volumetric flask. From this stock solution, respective K standards (20, 40, 60, 80, 100, 150, and 200 ppm) were prepared with 1 N ammonium acetate ( $\text{NH}_4\text{OAc}$ ) in 100 mL volumetric flask.

Soil (10 g) was shaken with 1 N  $\text{NH}_4\text{OAc}$  solution (50 mL) for 30 minutes on a reciprocating shaker at a speed of 200-300 rpm. After shaking, the suspension was passed through a filter with Whatman No. 1 filter paper and 50 ml volume was made with 1 N  $\text{NH}_4\text{OAc}$  solution.

The instrument was standardized with a sequence of prepared respective standards and a standard curve was made. Extractable K (ppm) was determined by recording the emission readings on flame photometer (Jenway PFP-7) by adjusting its wavelength at 767 nm.

Concentration of soluble K (mg kg<sup>-1</sup>) was calculated by the following formula:

$$\text{Soluble K (mg kg}^{-1}\text{)} = \frac{\text{K (mg kg}^{-1}\text{) from standard curve} \times V \times V_2}{\text{weight of soil sample (g)} \times V_1}$$

Where,

V = Volume of the soil extract used (mL)

V<sub>1</sub> = Volume of the soil extract used for the measurement (mL)

V<sub>2</sub> = Volume of the flask used for the measurement (mL)

## 2.4 Harvesting

At first, 1 m<sup>2</sup> area for each plot was harvested for accessing some agronomic, yield and nutrient parameters. After harvesting, total numbers of cobs per plant were counted from each plot along with non-productive cobs. By subtracting the non-productive from total number of cobs, productive cobs were obtained. From experimental plot, three plants were taken, and their height was calculated and measured from land surface to the plant tip and average value for plant height was measured in cm. The spike length of the same harvested plants was taken, and their average value was calculated. From collected cobs 100 grains weight were calculated manually and their total weight was calculated by using the weighing balance. From the experimental plot, plant biomass and yield were also calculated.

## 2.5 Plant nutrient analysis

### a. Preparation of samples

For full recovery of nutrients, all the shoots, grains and roots samples were wet digested by following the procedure of (Rashid, 1986). About 0.25 grams of oven-dried plant sample and 2.5 mL of nitric acid and per-chloric acid (HNO<sub>3</sub>–HClO<sub>4</sub>) (ratio 2:1) mixture were added in the 50 mL Pyrex conical flasks. These digestion flasks were kept overnight for complete dissolution of plant samples in added acid. Then, these flasks were kept on the hotplate at 150° C temperature till neutral appearance. After cooling, digested materials were diluted with distilled water to 30 mL volume. The digested materials were filtered by using Whatman No. 42 filter paper and preserved in airtight labeled plastic bottles after making 50 mL volume.

### b. Determination of phosphorus

Phosphorus concentration in digested shoot and grains samples was determined by following the vanadate-Molybdate method (also known as yellow color method) on an UV-visible spectrophotometer (CECIL CE 7400) (Chapman, 1961). For preparation of ammonium vanadate-molybdate reagent, ammonium molybdate (22.5 g) and ammonium vanadate (1.25 g) were dissolved in 400 mL and 300 mL distilled water, respectively. Then, both solutions were properly mixed in a 1 L volumetric flask and cooled at ambient



temperature. Then, concentrated HNO<sub>3</sub> (250 mL) was poured into the mixture. After cooling, 1 L volume of mixture was made with distilled water.

Volume of 5 mL from the filtered digest and vanadate molybdate which is a reagent was added into volumetric flask of 50 mL and volume was made using DI water. Then, the formulated samples were stayed for 20 minutes till development of stable yellow color. The same procedure was adopted for preparation of P standards (0-20 mg L<sup>-1</sup>) having all the reagents without sample. After calibrating the instrument by running the respective standards, P concentration in plant samples was determined at 410 nm wavelength. Actual P content in plant samples was estimated by plotting the instrument reading on the standard curve. Formula to determine the P concentration in (mg L<sup>-1</sup>):

$$P \text{ (mg L}^{-1}\text{)} = \frac{P \text{ (mg L}^{-1}\text{) from standard curve} \times V1 \times 100}{\text{weight of soil sample (g)} \times V2 \times 10000}$$

Where,

V1 = Total volume of plant digest (mL)

V2 = Volume of the plant digest which is used for the measurement (mL)

### c. Determination of potassium

Potassium concentration in digested plant samples was determined by flame photometer (Jenway PFP-7). The instrument was calibrated by running the respective K standards (0, 50, 100, 150, 200, and 250 mg L<sup>-1</sup>). Shoot samples were diluted up to 1000 times before running on the instrument due to high K concentration.

### 2.6 Microbial Biomass Carbon

Chloroform fumigation extraction procedure was used to measure the carbon content of the soil's microbial biomass. (Vance *et al.*, 1987). Two soil samples of amount 5 g were taken from each replicate, one for fumigation and other for the non- fumigation.

The fumigated and the non-fumigated samples, both were mixed in 20 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> solution then shaken for 30 min at 350 rpm with electric shaker. After that 4 ml filtrate was taken and added 5 mL H<sub>2</sub>SO<sub>4</sub>, 1 mL of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 2 to 3 drops of phenanthroline indicator and then titrated with 0.0337 M ferrous ammonium sulfate. Non-fumigated samples are also used for DOC determination.

Three blanks (only K<sub>2</sub>SO<sub>4</sub> filtrated without soil extracts) without H<sub>2</sub>SO<sub>4</sub> were titrated too to the endpoint which is color red. Chloroform role is to lyse microbes and make their contents release into the soil solution.

Differentiation between the fumigated and the non-fumigated subsamples is soil MBC.

The conversion factor for soil MBC is 0.45 (Joergensen and Mueller, 1996).

$$\text{microbial biomass carbon (mg C kg}^{-1} \text{ soil)} = \frac{(\text{fumigated C} - \text{nonfumigated C})}{K_{EC}}$$

Here,

$$\text{Fumigated or Non fumigated C} = \frac{(V_1 - V_2) \times 0.003 \times 0.033}{\text{wt. of oven dry soil}} \times \frac{(\text{Vol. of K}_2\text{SO}_4 \text{ used})}{\text{Vol. of soil extract}} \times 100 \times 1000$$

$$K_{ec} = 0.45 \text{ (Conversion factor of the microbial C flush into MBC)}$$

Where,

$V_1$  = Volume of ferrous ammonium sulfate used for blank

$V_2$  = Volume of ferrous ammonium sulfate used for sample

0.033 = Molarity of ferrous ammonium sulfate solution.

0.003 = mili equivalent of C

100 = conversion factor of OM into %

## 2.7 Extracellular enzymes activity

Fluorogenically labeled substrates technique was used to measure extracellular enzymes actions. (Pritsch *et al.*, 2004) (Sanaullah *et al.*, 2011). For measuring of acid phosphatase,  $\beta$ -glucosidase, alkaline phosphatase, chitinase and the leucine-aminopeptidase enzymes, four fluorogenic enzyme substrates MUF- $\beta$ -D-glucopyranoside 4-MUF-Phosphate, 4-MUF-N-acetyl- $\beta$ -D-glucosaminide and L-Leucine-7-AMC used respectively for their specific enzymes. A list of enzymes, respective substrates and possibly degradable mixtures is provided in Tab. 2.7. The MUF-substrates were mixed in 2-methoxyethanol and further diluted with sterile water to make working concentration (Hoppe, 1983).

**Table 2.7: Functions and substrates for the enzymes activity estimation**

Enzymes	Substrate	Functions
<b>Carbon cycle</b>		
$\beta$ -Glucosidase	4-MUF- $\beta$ -glucopyranoside	Cellulose degradation
Chitinase	4-MUF-N-acetyl- $\beta$ -D-glucosaminide	Chitin degradation
<b>Phosphorous cycle</b>		
Acid phosphatase	4-MUF-Phosphate	Phosphorous cycle
<b>Nitrogen cycle</b>		
Leucine-aminopeptidase	L-Leucine-7-AMC	Peptides degradation

4-MUF = 4-methylumbelliferone and 7-AMC = 7-amino-4-methyl coumarin.

Soil suspension was made in the centrifuge tube by taking 0.5 g soil (dry weight equivalent) with 50 mL sterile water and then shaken for 30 min at 350 rpm by a mechanical shaker. For each enzyme 50  $\mu$ L of its soil suspension was taken by stirring it

on a magnetic shaker in 96 wells microplate. The MES buffer of 50  $\mu\text{L}$  for the enzymes (i.e.,  $\beta$ -Glucosidase, Acid Phosphatase and Chitinase), 1M NaOH for Alkaline Phosphatase with TRIZMA buffer (50  $\mu\text{L}$ ) for the Leucine- Aminopeptidase depending on substrate was pipetted into wells. Substrate solution 100  $\mu\text{L}$  MUF for the MES buffer while AMC for TRIZMA buffer was added as well. Fluorescence was assessed in the microplates within 2 to 3 min in the microplate reader (Biotech Multi Mode Reader) at excitation/emission wave-length of 360/460 nm after a time of 0 and 120 min. The files were stored in the form of an excel spreadsheet. Before next measurement the soil and standard plates remained at room temperature before each reading plates were shaken for 1-2 min. Enzyme activities were expressed as MUF or AMC release in  $\text{nmol g}^{-1} \text{h}^{-1}$ . Michaelis–Menten equation was used to determined enzyme activity.

$$\text{Enzymes activity } (= \frac{(\text{Fs}-\text{Fe}) \times 0.936 \text{ or } 1.801 \times \text{V1 } (\mu\text{L})}{\text{V2 } (\mu\text{L}) \times 1000 \times \text{dry soil wt. (g)} \times \text{incubation time (h)}}$$

Where,

- Fs: fluorescence of the sample
- Fe: fluorescence of an empty well
- 0.936: scale obtained from standard MUF
- 1.801: scale obtained from standard AMC
- V1: total soil suspension volume (50  $\mu\text{L}$ )
- V2: Volume of soil suspension taken for analysis ( $\mu\text{L}$ )
- 1000: conversion factor (p Mol – n Mol)

## 2.8 Statistical analysis

All the recorded data were subjected to two-way analysis of variance (ANOVA) and the significant differences were measured by the least significant difference (LSD) test a 5% probability level ( $p < 0.05$ ) using the Statistix 8.1 software.

## 3. RESULTS AND DISCUSSIONS

### 3.1. Basic soil characteristics

**Table 3.1: Soil physicochemical characteristics**

zSoil characteristics	Topsoil (0-15 cm)	Subsoil (15-30 cm)	Unit
pH	8.03±0.06	7.70±0.10	
EC	1.38±0.03	1.11±0.02	dS m <sup>-1</sup>
OM	0.90±0.09	0.48±0.04	%
Total P	16.77±2.52	10.90±1.62	mg kg <sup>-1</sup>
Soluble K	105±6.68	74.07±0	mg kg <sup>-1</sup>
Total N	0.04±0	0.03±0	%
Sand	26.67±1.4	26.33±2.3	%
Silt	45±0	47±0.8	%
Clay	28.33±1.4	26.67±1.4	%
Texture	Clay loam	Loam	

### 3.2. Wheat residue and its biochar characteristics

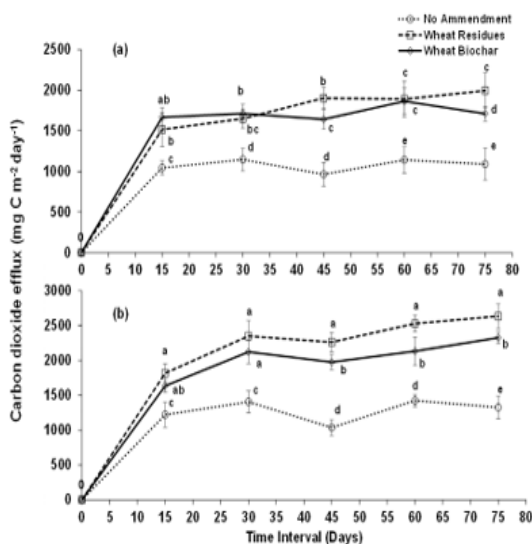
**Table 3.2: Wheat residue and its biochar physicochemical characteristics**

Characteristics	Wheat Residue	Wheat Biochar	Unit
pH	8.33±0.06	8.33±0.12	
EC	0.98±0.05	2.67±0.03	dS m <sup>-1</sup>
Total P	0.25±0.008	0.26±0.004	%
Soluble K	1.1±0.06	1.24±0.2	%
Total N	0.38±0.02	0.50±0.03	%
C	15.1±0.12	28±2.1	%

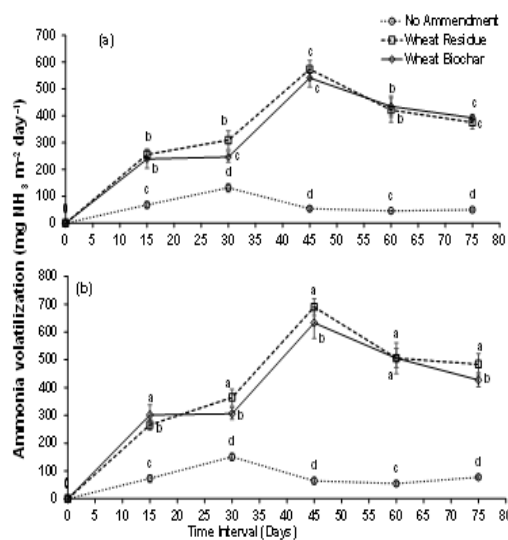
### 3.3. Gaseous emissions

#### 3.3.1. Carbon dioxide efflux and Ammonia volatilization

Results showed that CO<sub>2</sub> efflux was higher under application of wheat residues as incorporation at 75<sup>th</sup> day (2634±179.33 mg C m<sup>-2</sup> day<sup>-1</sup>) as compared to no amendment. Application of wheat biochar as incorporation also increased CO<sub>2</sub> efflux (2325±85.63 mg C m<sup>-2</sup> day<sup>-1</sup>) as compared to its mulching and no amendment as shown in Fig.3.3.1(b). The CO<sub>2</sub> efflux was also influenced positively by application of wheat residues as mulching as compared to no amendment as shown in Fig.3.3.1(a).



**Figure.3.3.1: Impact of wheat residue and wheat biochar under mulching (a) and incorporation (b) on CO<sub>2</sub> efflux. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is denoted as mean±SD (n=4)**



**Figure.3.3.2: Impact of wheat residue and wheat biochar under mulching (a) and incorporation (b) on NH<sub>3</sub> volatilization. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is denoted as mean±SD (n=4)**

Ammonia volatilization was higher under application of wheat residue as incorporation at 45<sup>th</sup> day ( $689.99 \pm 30.3 \text{ mg NH}_3 \text{ m}^{-2} \text{ day}^{-1}$ ) as compared to its mulching and no amendment as shown in Fig.3.3.2(b). It was also increased by application of wheat biochar as incorporation on 45<sup>th</sup> day ( $633.3 \pm 56.83 \text{ mg NH}_3 \text{ m}^{-2} \text{ day}^{-1}$ ) as compared to no amendment. Mulching of wheat residue and wheat biochar also influenced positively on  $\text{NH}_3$  volatilization as compared to no amendment as shown in Fig.3.3.2(a).

### **3.4. Agronomic parameters**

#### **3.4.1. Number of plants per meter square**

Results were non-significant for number of plants per meter square under both methods of application of wheat residue and wheat biochar as mulching and incorporation ( $10 \pm 0$ ). Number of plants remained same.

#### **3.4.2. Number of cobs per plant**

Application of wheat residue and wheat biochar as mulching and incorporation had non-significant impact on number of cobs per plant in all treatments. However, number of cobs per plants was highest in no amendment in both application methods ( $10 \pm 0.82$ ) as shown in Fig.4.4.

#### **3.4.3. Plant height**

Application of wheat biochar as incorporation significantly ( $p < 0.05$ ) increased plant height ( $92.5 \pm 7.3 \text{ cm}$ ) as compared to its mulching and no amendment. Plant height was non-significant under application of wheat residue as incorporation and mulching. Results in control were also non-significant.

#### **3.4.4. Total plant fresh biomass**

Application of wheat biochar as incorporation significantly ( $p < 0.05$ ) increased total biomass of fresh plants ( $7 \pm 0.3 \text{ g}$ ) whereas application of wheat residue as mulching and incorporation had non-significant impact on total biomass of fresh plants but as compared to mulching of wheat biochar, incorporation of wheat residue also significantly increased total biomass of fresh plants. Results were also non-significant in no amendment.

#### **3.4.5. Dry plant biomass**

Application of wheat residue and wheat biochar in both application practices (mulching and incorporation) had non-significant impact on dry plant biomass. However dry plant biomass was seen highly increased in incorporation of wheat residue and wheat biochar as compared to mulching and control. Results were also non-significant in no amendment.

#### **3.4.6. Total cob weight with ears**

Application of wheat residue and wheat biochar in both practices mulching and incorporation had non-significant impact on total weight of cob with ears. Results were also non-significant in no amendment.

### 3.4.7. Total cob weight without ears

Application of wheat residue and its biochar as mulching and incorporation had non-significant impact on total weight of cobs without ears. Results were also non-significant in no amendment.

### 3.4.8. Grain rows per cob

Application of wheat residue and its biochar in both application methods (incorporation and mulching) had non-significant impacts on number of grain rows per cob.

### 3.4.9. Diameter of cobs

Application of wheat biochar as incorporation significantly ( $p < 0.05$ ) increased the diameter of cobs ( $50.7 \pm 0.31$  cm) as compared to mulching. Incorporation of wheat residue also significantly increased the diameter of cob ( $51 \pm 0.5$  cm) as compared to no amendment.

### 3.4.10. Length of cob

Application of wheat residue as mulching significantly ( $p < 0.05$ ) increased length of cob ( $17 \pm 0.63$  cm) as compared to application of wheat biochar and no amendment. Length of cob by applying wheat biochar as mulching was also increased ( $16.8 \pm 0.83$  cm) as compared to no amendment and incorporation of wheat biochar.

## 3.5. Yield parameters

### 3.5.1. 1000 Grains weight and Total grain weight yield

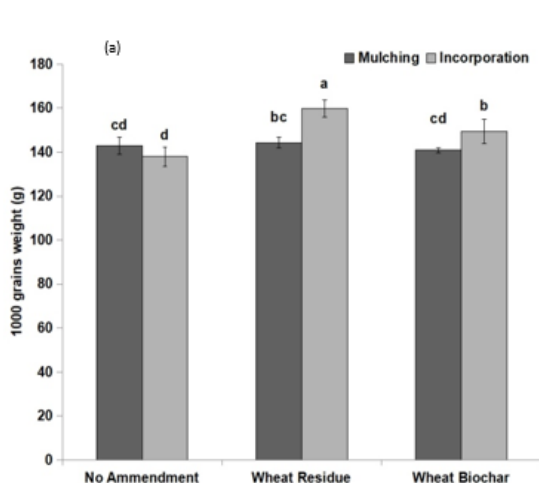


Figure.3.5.1(a): Impact of wheat residue and wheat biochar under mulching and incorporation on 1000 grains weight. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is characterized as mean $\pm$ SD (n=4)

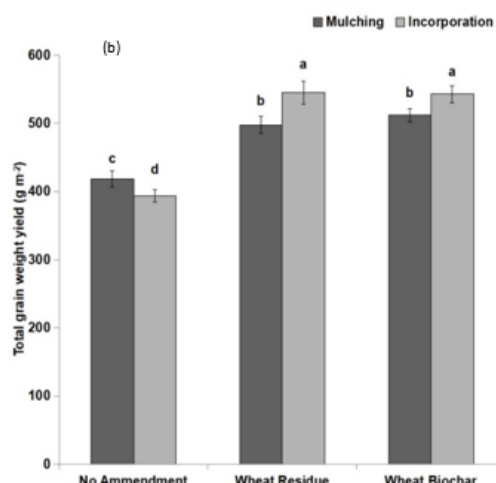


Figure.3.5.1(b): Impact of wheat residue and wheat biochar under mulching and incorporation on total grain weight yield. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is characterized as mean $\pm$ SD (n=4)



Application of wheat residue as incorporation significantly ( $p < 0.05$ ) increased weight of 1000 grains ( $160 \pm 3.94$  g) as compared to control and mulching. Application of wheat biochar as incorporation also significantly increased weight of 1000 grains ( $150 \pm 5.7$  g) as compared to no amendment and mulching as shown in Fig.3.5.1 (a).

Application of wheat residue as incorporation significantly ( $p < 0.05$ ) increased total grain weight yield ( $545 \pm 17.09$ ) as compared to mulching. Application of wheat biochar as incorporation significantly increased total grain weight yield ( $543 \pm 12.5$ ) as compared to mulching and no amendment as shown in Fig.3.5.1(b).

### 3.6. Plant nutrient analysis

#### 3.6.1. Phosphorus in grain and Shoot

Application of wheat biochar as incorporation significantly ( $p < 0.05$ ) increased P in grain ( $0.21 \pm 0.03\%$ ) as compared to no amendment. Mulching of wheat residue ( $0.2 \pm 0.02\%$ ) and its incorporation ( $0.2 \pm 0.02\%$ ) also increased P in grain as compared to no amendment as shown in Fig. 3.6.1(a).

Application of wheat biochar as incorporation significantly ( $p < 0.05$ ) increased P in shoot ( $0.30 \pm 0.003\%$ ) as compared to its mulching ( $0.25 \pm 0.04\%$ ). Application of wheat residue as mulching ( $0.24 \pm 0.04\%$ ) and incorporation ( $0.23 \pm 0.04\%$ ) also increased P in shoot as compared to no amendment as shown in Fig. 3.6.1(b).

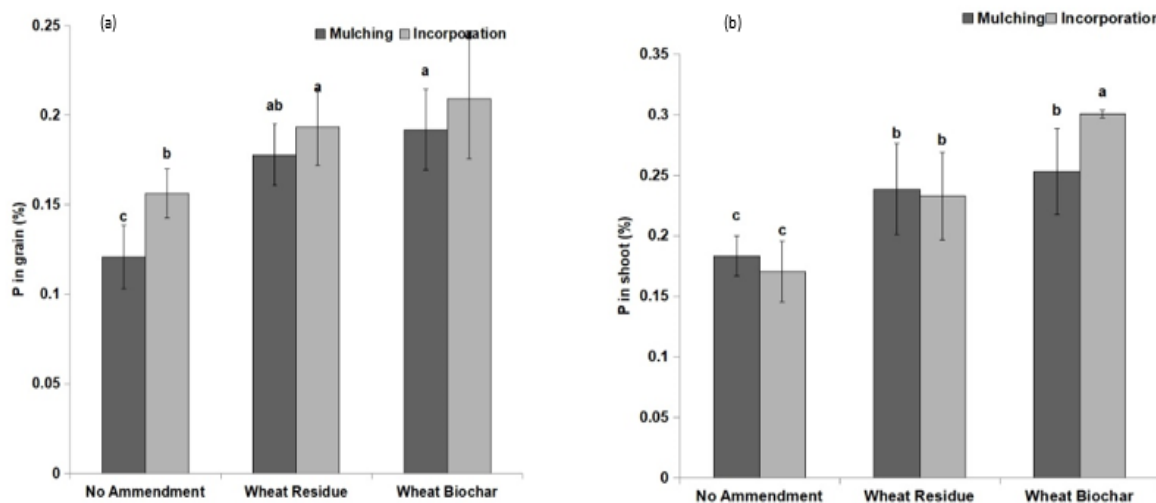


Figure.3.6.1(a): Impact of wheat residue and wheat biochar under mulching and incorporation on phosphorus in grain. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is exemplified as mean $\pm$ SD (n=4)

Figure.3.6.1(b): Impact of wheat residue and wheat biochar under mulching and incorporation on phosphorus in shoot. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is exemplified as mean $\pm$ SD (n=4)

### 3.6.2. Potassium in plant

#### 3.6.2.a. Potassium in grain

Potassium in grain was notably ( $p < 0.05$ ) increased by incorporation of wheat residue ( $2.44 \pm 0.8\%$ ) and wheat biochar ( $2.5 \pm 1.8\%$ ) as compared to mulching of wheat residue ( $1.6 \pm 0.2\%$ ) and wheat biochar ( $1.81 \pm 0.3\%$ ) and no amendment.

#### 3.6.2.b. Potassium in shoot

Application of wheat residue as incorporation ( $2.865909 \pm 0.216932\%$ ) and wheat biochar as mulching ( $2.734596 \pm 0.223458\%$ ) significantly ( $p < 0.05$ ) increased potassium in shoot as compared to no amendment. The percentage of potassium was highest in application of wheat residue as incorporation ( $2.9 \pm 0.21\%$ ).

### 3.7. Soil nutrient analysis

#### 3.7.1. Soil phosphorus and Soil potassium

Application of wheat residue as incorporation significantly ( $p < 0.05$ ) increased extractable phosphorus in topsoil ( $16.31 \pm 2.2 \text{ mg kg}^{-1}$ ) in contrast to its mulching and no amendment. Whereas, in no amendment and application of wheat biochar in both treatments, mulching ( $14.8 \pm 2.92 \text{ mg kg}^{-1}$ ) and incorporation ( $15.9 \pm 1.41 \text{ mg kg}^{-1}$ ) also significantly increased P in topsoil as compared to no amendment as shown in Fig.3.7.1(a).

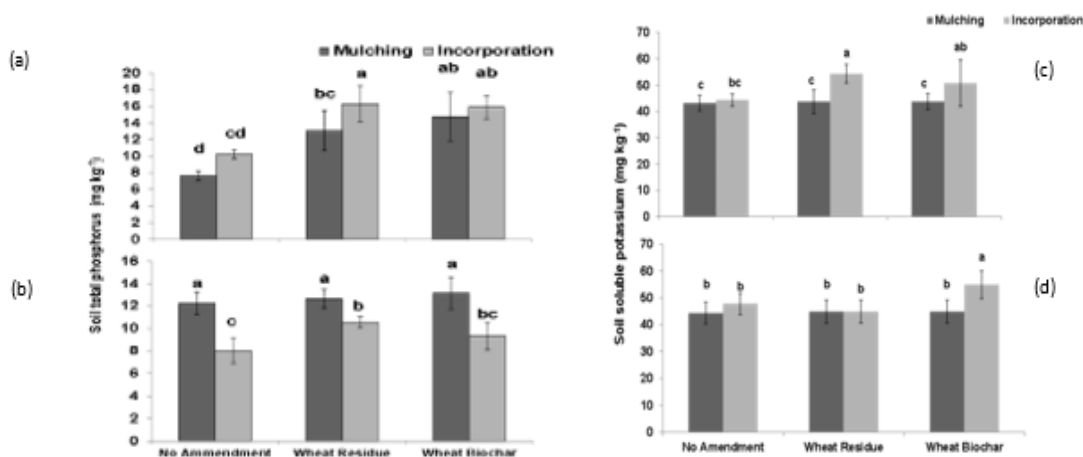


Figure.3.7.1 (a)(b): Impact of wheat residue and wheat biochar under mulching and incorporation on soil phosphorus. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is denoted as mean $\pm$ SD (n=4)

Figure.3.7.1 (c)(d): Impact of wheat residue and wheat biochar under mulching and incorporation on soil potassium. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is denoted as mean $\pm$ SD (n=4)

Phosphorus in subsoil, significantly ( $p < 0.05$ ) improved by application of wheat residue as mulching ( $12.6 \pm 0.9 \text{ mg kg}^{-1}$ ) and its biochar as mulching ( $13.2 \pm 1.43 \text{ mg kg}^{-1}$ ) as compared to incorporation of wheat residue ( $10.56 \pm 0.5 \text{ ppm}$ ) and wheat biochar ( $9.33 \pm 1.22 \text{ mg kg}^{-1}$ ). In no amendment, results were also highly significant as shown in Fig.3.7.1(b).

Application of wheat residue as incorporation significantly ( $p < 0.05$ ) increased K in topsoil ( $54 \pm 3.6 \text{ mg kg}^{-1}$ ) as compared to its mulching and no amendment. Whereas application of wheat biochar as incorporation ( $50.82 \pm 8.89 \text{ mg kg}^{-1}$ ) also significantly increased K in topsoil as compared to mulching and no amendment as shown in Fig.3.7.1(c).

Potassium in subsoil, significantly ( $p < 0.05$ ) enhanced by application of wheat biochar as incorporation ( $55 \pm 5.21 \text{ mg kg}^{-1}$ ) as compared to its mulching. Mulching and incorporation of wheat residue had non-significant impact on soil K as shown in Fig.3.7.1(d).

### 3.7.2. Soil nitrogen and Microbial biomass carbon

Application of wheat biochar as incorporation significantly ( $p < 0.05$ ) increased N in soil ( $0.15 \pm 0\%$ ) as compared to its mulching ( $0.11 \pm 0\%$ ) and no amendment. Whereas application of wheat residue as incorporation ( $0.15 \pm 0.02\%$ ) and mulching ( $0.16 \pm 0.02\%$ ) also significantly increased N in soil as compared no amendment as shown in Fig.3.7.2(a).

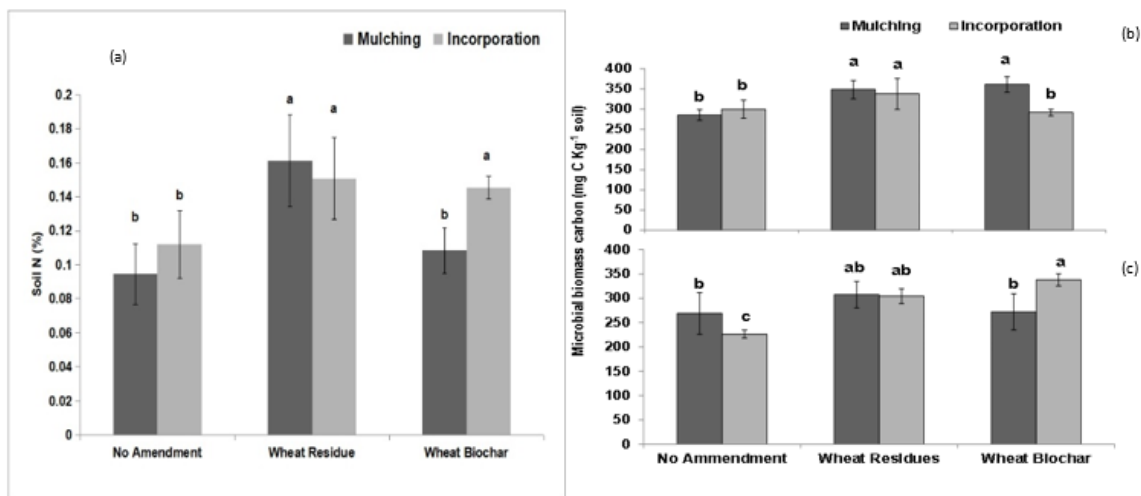


Figure.3.7.2(a): Impact of wheat residue and wheat biochar under mulching and incorporation on soil nitrogen. Total six treatments were maintained. Data was analyzed under two-way ANOVA. Data is represented as mean±SD (n=4)

Figure.3.7.2(b)(c): Impact of wheat residue and wheat biochar under mulching and incorporation on soil microbial biomass carbon. Total six treatments were maintained. Data was analyzed under two-way ANOVA. Data is represented as mean±SD (n=4)

MBC in topsoil was significantly ( $p < 0.05$ ) enhanced by applying wheat biochar as mulching ( $360.9 \pm 19.3 \text{ mg C Kg}^{-1} \text{ soil}$ ) as compared to its incorporation ( $291.6 \pm 8.1 \text{ mg C Kg}^{-1} \text{ soil}$ ) as shown in Fig.3.7.2(b).

Kg<sup>-1</sup> soil). Application of wheat residue as mulching (348.33±22.6 mg C Kg<sup>-1</sup> soil) and incorporation (337.8±38.7 mg C Kg<sup>-1</sup> soil) also increased microbial biomass carbon in topsoil as compared to amendment as shown in Fig.3.7.2(b).

Microbial biomass carbon in subsoil was pointedly (p<0.05) improved by applying wheat biochar as incorporation (337.9±12.20 mg C Kg<sup>-1</sup> soil) as compared to its mulching (272.01±37.3 mg C Kg<sup>-1</sup> soil). Application of wheat residues as mulching (307.64±27.1 mg C Kg<sup>-1</sup> soil) and incorporation (304.4±15.5 mg C Kg<sup>-1</sup> soil) also increased microbial biomass carbon in subsoil as compared to no amendment as shown in Fig.3.7.2(c).

### 3.8. Extracellular enzyme activity

#### 3.8.1. β-Glucosidase and Chitinase

Application of wheat biochar as incorporation had significant (p<0.05) impact on β-glucosidase activity (88.26±16.82 n M g<sup>-1</sup> soil hr<sup>-1</sup>) in contrast with its mulching (60.03±8.53 n M g<sup>-1</sup> soil hr<sup>-1</sup>). Wheat residues had non-significant impact in both of the treatments, mulching (91.9±17.83 n M g<sup>-1</sup> soil hr<sup>-1</sup>) and incorporation (84.71±12.6 n M g<sup>-1</sup> soil hr<sup>-1</sup>). In no amendment results were also non-significant as shown in Fig.3.8.1(a).

Application of wheat residues as mulching had significant (p<0.05) impact on chitinase activity (564.83±96.01 n M g<sup>-1</sup> soil hr<sup>-1</sup>) in contrast with its incorporation (377.27±71.01 n M g<sup>-1</sup> soil hr<sup>-1</sup>). Chitinase action was also considerably enhanced by the application of wheat biochar as mulching (473.43±30.5 n M g<sup>-1</sup> soil hr<sup>-1</sup>) in contrast with its incorporation (375.61±14.54 n M g<sup>-1</sup> soil hr<sup>-1</sup>). Results were also significant where there was no amendment as shown in Fig.3.8.1(b).

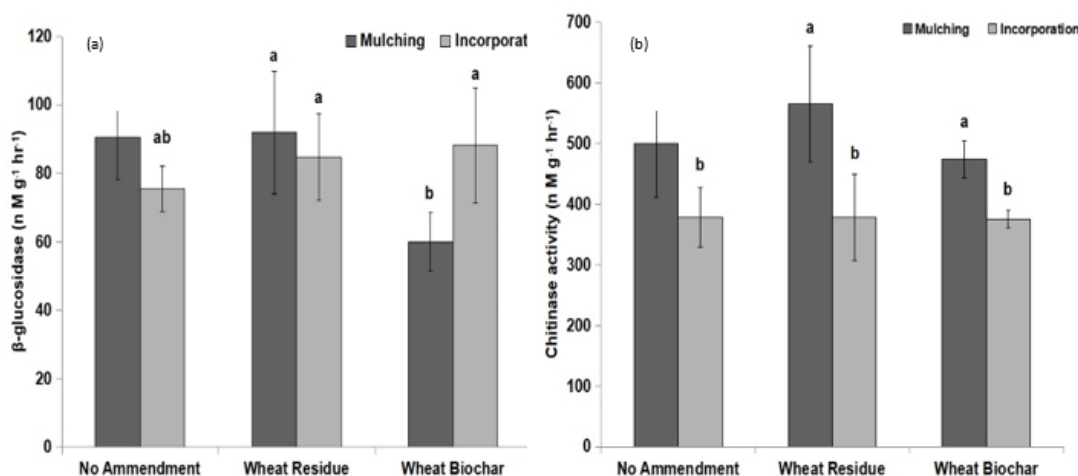


Figure.3.8.1(a): Impact of wheat residue and wheat biochar under mulching and incorporation on β-glucosidase activity. Total six treatments were maintained. Data was analyzed under two-way ANOVA. Data is represented as mean±SD (n=4)

Figure.3.8.1(b): Impact of wheat residue and wheat biochar under mulching and incorporation on chitinase activity. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is represented as mean±SD (n=4)

### 3.8.2. Leucine amino peptidase and Acid phosphatase

Application of wheat biochar as incorporation significantly ( $p < 0.05$ ) decreased leucine amino peptidase activity ( $4220.3 \pm 634.61 \text{ n M g}^{-1} \text{ soil hr}^{-1}$ ) as compared to no amendment. Mulching and incorporation of wheat residues and wheat biochar had non-significant impact on leucine amino peptidase activity as shown in Fig.3.8.2(a).

Application of wheat biochar as incorporation had highly significant ( $p < 0.05$ ) impact on acid phosphatase activity ( $412.63 \pm 81.8 \text{ n M g}^{-1} \text{ soil hr}^{-1}$ ) as compared to its mulching ( $305.8 \pm 55.3 \text{ n M g}^{-1} \text{ soil hr}^{-1}$ ).

Activity of Acid phosphatase was also enhanced by the application of wheat residue as mulching ( $383.8 \pm 45.8 \text{ n M g}^{-1} \text{ soil hr}^{-1}$ ) in contrast with its incorporation ( $333.02 \pm 65.2 \text{ n M g}^{-1} \text{ soil hr}^{-1}$ ). Results were non-significant in no amendment as shown in Fig.3.8.2(b).

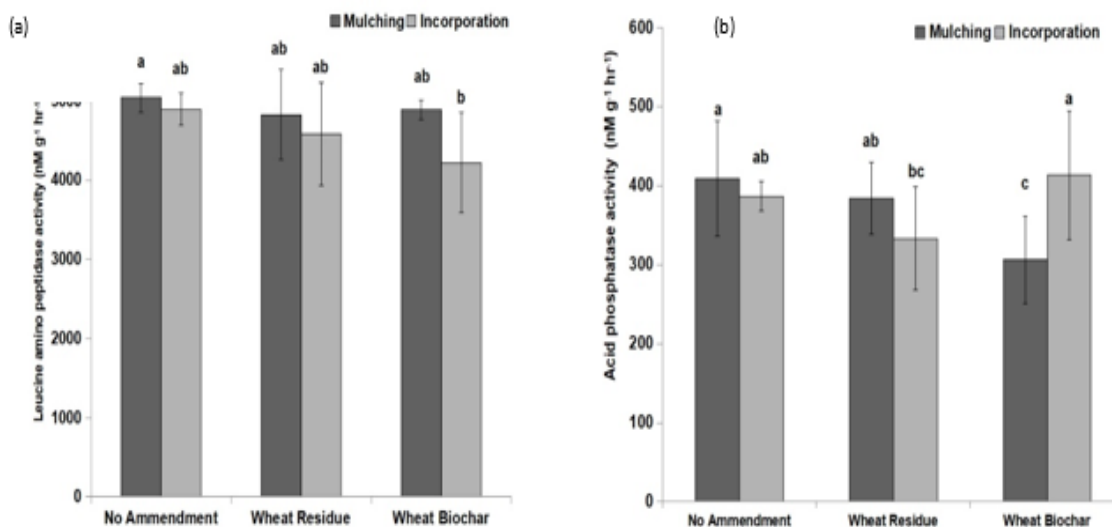


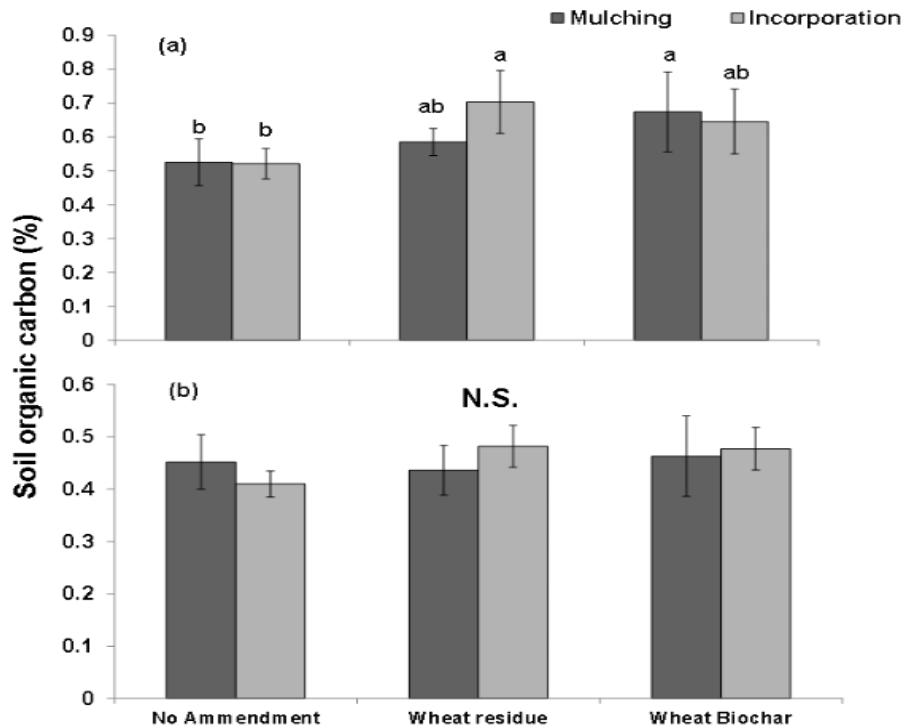
Figure.3.8.2(b): Impact of wheat residue and wheat biochar under mulching and incorporation on leucine amino peptidase activity. Total six treatments were maintained. Data was analyzed under two-way ANOVA. Data is represented as mean $\pm$ SD (n=4)

Figure.3.8.2(a): Impact of wheat residue and wheat biochar under mulching and incorporation on acid phosphatase activity. Total six treatments were maintained. Data was analyzed under two-way ANOVA. Data is represented as mean $\pm$ SD (n=4)

### 3.8.3. Soil organic carbon

SOC was significantly ( $p < 0.05$ ) increased by application of wheat residue as incorporation ( $0.7034 \pm 0.1\%$ ) and wheat biochar as mulching ( $0.6743 \pm 0.12\%$ ) as compared to no amendment as shown in Fig.3.8.3(a).

Results for SOC in subsoil were non-significant as shown in Fig.3.8.3(b).



**Figure 3.8.3: Impact of wheat residue and wheat biochar under mulching and incorporation on soil organic carbon dynamics. A total of six treatments were maintained. Data was analyzed under two-way ANOVA. Data is characterized as mean±SD (n=4)**

#### 4. DISCUSSION

Incorporation of wheat residues and wheat biochar had significant influence on SOC and CO<sub>2</sub> efflux (Xiao *et al.*, 2019). Incorporation of wheat residue and its biochar had higher CO<sub>2</sub> efflux as compared to mulching and no amendment and it may be due to biotic factors including microbial respiration and higher SOC decomposition in topsoil and abiotic factors including temperature which increases microbial respiration under incorporation (Fortin *et al.*, 1996). It may be also due to higher C substrate from root exudates in upper layer of soil which influence microbial community (Fierer *et al.*, 2003). Application of wheat residue enhanced the CO<sub>2</sub> efflux as compared to treatments with no residue and biochar amendment because crop residue act as substrate for microbial activity which increases soil respiration (Ghimire *et al.*, 2017). Soil microbes utilize the labile C fraction in residue and biochar which enhanced the rate of C mineralization (Sharma *et al.*, 2019). Decomposition rate is significantly influenced by C:N ratio of added residue and biochar. Residue having narrow C:N ratio facilitates mineralization while wider C:N ratio causes immobilization. The increase in CO<sub>2</sub> efflux in later phase of experiment was may be due to increase in availability of easily decomposable materials



(Sarkar *et al.*, 2020). It was also suggested that addition of mineral fertilizers enhances the CO<sub>2</sub> emissions (Cheng-Fang *et al.*, 2012). Residue and biochar mineralization is also influenced by climatic factors. Increase in temperature at higher moisture level enhances the rate of mineralization (Wang *et al.*, 2010). Incorporation of wheat residue increased the emissions of CO<sub>2</sub> due to more surface area explored with soil particles and microbes which promotes rate of decomposition, but our results showed highest efflux with incorporation of wheat residue, may be due to higher moisture content under mulching which increases microbial activity.

Ammonia volatilization is usually contemplated as one of key nitrogen loss alleyways from the agricultural land. Particularly in areas with alkaline soils, nitrogen loss via ammonia volatilization can be considered for >30% of the fertilizer. It may be also due to addition of N based fertilizers and environmental factors, high temperature and moisture effects the labile and recalcitrant pools of N (Belay-Tedla *et al.*, 2009). Wheat residue and its biochar as an organic amendment enhanced NH<sub>3</sub> volatilization as compared to control.

## References

- 1) Akram, H.M.B., Ullah, I., Nabi, H.G., Shahzad, N., Ahmad, M. and Farzand, A., 2023. Optimization of plant spacing for yield improvement in hybrid maize using DSSAT. *Agrobiological Records* 14: 37-49.
- 2) Alvarenga, P., Carneiro, J.P., Fangueiro, D., Cordovil, C.M. and Bernal, M.P., 2020. Managing organic amendments in agroecosystems to enhance soil carbon storage and mitigate climate change. In *Climate change and soil interactions*. 89-141. Elsevier.
- 3) Anderson, J.P. 1983. Soil respiration. *Methods of soil analysis: part 2 chemical and microbiological properties*. 9:831-871.
- 4) Belay-Tedla, A., X. Zhou, B. Su, S. Wan and Y. Luo. 2009. Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the us great plains subjected to experimental warming and clipping. *Soil Biol. Biochem.* 41:110-116.
- 5) Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analyses of soils 1. *Agron. J.* 54:464-465.
- 6) Bremner, J. and D.R. Keeney. 1965. Steam distillation methods for determination of ammonium, nitrate and nitrite. *Anal. Chim. Acta.* 32:485-495.
- 7) Cheng-Fang, L., Z. Dan-Na, K. Zhi-Kui, Z. Zhi-Sheng, W. Jin-Ping, C. Ming-Li and C. Cou-Gui. 2012. Effects of tillage and nitrogen fertilizers on ch<sub>4</sub> and co<sub>2</sub> emissions and soil organic carbon in paddy fields of central China. *PLOS One.* 7:346-412.
- 8) Das, R., Ghosh, A., Das, S., Basak, N., Singh, R., Priyanka and Datta, A., 2021. Soil carbon sequestration for soil quality improvement and climate change mitigation. *ACCUS.* 57-81.
- 9) Elbasiouny, H., El-Ramady, H., Elbehiry, F., Rajput, V.D., Minkina, T. and Mandzhieva, S., 2022. Plant nutrition under climate change and soil carbon sequestration. *Sustainability.* 14(2): 914.
- 10) Farzand, A., Akram, H.M.B., Ahmad, A., Aslam, Z., Bellitürk, K., Büyükfiliz, F. and Sözübek, B., 2023. Plant and Soil Data Management Via Intelligent Agricultural Machinery and Field Robots. (Climate Change and Soil-Plant-Environment Interactions. Editors: K. Bellitürk, A. Çelik, M. Kiliç, and F. Büyükfiliz). IKSAD Publishing House. ISBN: 978-625-367-101-3, Chapter 1: 9-36, Print Date: 1 June, 2023, Ankara.

- 11) Fierer, N., J.P. Schimel and P.A. Holden. 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biol. Biochem.* 35:167-176.
- 12) Fortin, M.C., P. Rochette and E. Pattey. 1996. Soil carbon dioxide fluxes from conventional and no-tillage small-grain cropping systems. *Soil Science Society of America Journal.* 60:1541-1547.
- 13) Ghimire, B., R. Ghimire, D. VanLeeuwen and A. Mesbah. 2017. Cover crop residue amount and quality effects on soil organic carbon mineralization. *Sustain.* 9:231-246.
- 14) Hoppe, H.-G. 1983. Significance of exoenzymatic activities in the ecology of brackish water: Measurements by means of methylumbelliferyl-substrates. *Marine Eco. Progress seri.* 21:299-308.
- 15) Hou, D., Bolan, N.S., Tsang, D.C., Kirkham, M.B. and O'connor, D., 2020. Sustainable soil use and management: An interdisciplinary and systematic approach. *Sci. Total Environ.* 729: 138961.
- 16) Hussain, S., Hussain, S., Guo, R., Sarwar, M., Ren, X., Krstic, D., Aslam, Z., Zulifqar, U., Rauf, A., Hano, C. and El-Esawi, M.A., 2021. Carbon sequestration to avoid soil degradation: A review on the role of conservation tillage. *Plants*, 10(10): 2001.
- 17) Joergensen, R.G. and T. Mueller. 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the ken value. *Soil Biol. Biochem.* 28:33-37.
- 18) Kuzyakov, Y. and W. Cheng. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biol. Biochem.* 33:1915-1925.
- 19) Lal, R., Eckert, D.J., Fausey, N.R. and Edwards, W.M., 2020. Conservation tillage in sustainable agriculture. *Sustain. Agric. Sys.* 203-225. CRC Press.
- 20) Littrell, J., Xu, S., Omondi, E., Saha, D., Lee, J. and Jagadamma, S., 2021. Long-term organic management combined with conservation tillage enhanced soil organic carbon accumulation and aggregation. *SSSAJ.* 85(5):1741-1754.
- 21) M. Tahat, M., M. Alananbeh, K., A. Othman, Y. and I. Leskovar, D., 2020. Soil health and sustainable agriculture. *Sustainability*, 12(12):4859.
- 22) Maenhout, P., Di Bene, C., Cayuela, M.L., Diaz-Pines, E., Govednik, A., Keuper, F., Mavsar, S., Mihelic, R., O'Toole, A., Schwarzmann, A. and Suhadolc, M., 2024. Trade-offs and synergies of soil carbon sequestration: Addressing knowledge gaps related to soil management strategies. *European Journal of Soil Science*, 75(3):13515.
- 23) Mandal, A., Dhaliwal, S.S., Mani, P.K. and Toor, A.S., 2021. Conservation agricultural practices under organic farming. *Advances in Organic Farming.* 17-37. Woodhead Publishing.
- 24) Martínez-Mena, M., Carrillo-López, E., Boix-Fayos, C., Almagro, M., Franco, N.G., Díaz-Pereira, E., Montoya, I. and De Vente, J., 2020. Long-term effectiveness of sustainable land management practices to control runoff, soil erosion, and nutrient loss and the role of rainfall intensity in Mediterranean rainfed agroecosystems. *Catena.* 187:104352.
- 25) Nazir, M.J., Li, G., Nazir, M.M., Zulfiqar, F., Siddique, K.H., Iqbal, B. and Du, D., 2024. Harnessing soil carbon sequestration to address climate change challenges in agriculture. *Soil Till. Res.* 237:105959.
- 26) Pearsons, K.A., Omondi, E.C., Zinati, G., Smith, A. and Rui, Y., 2023. A tale of two systems: Does reducing tillage affect soil health differently in long-term, side-by-side conventional and organic agricultural systems?. *Soil and Tillage Research*, 226:105562.
- 27) Pritsch, K., S. Raidl, E. Marksteiner, H. Blaschke, R. Agerer, M. Schloter and A. Hartmann. 2004. A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system. *J. Microbio. Methods.* 58:233-241.

- 28) Rahman, G.M., Rahman, M.M., Alam, M.S., Kamal, M.Z., Mashuk, H.A., Datta, R. and Meena, R.S., 2020. Biochar and organic amendments for sustainable soil carbon and soil health. *Carbon and nitrogen cycling in soil*. 45-85.
- 29) Rastogi, M., Verma, S., Kumar, S., Bharti, S., Kumar, G., Azam, K. and Singh, V., 2023. Soil health and sustainability in the age of organic amendments: A review. *Int. J. Environ. Clim.*, 13(10):2088-2102.
- 30) Rodrigues, C.I.D., Brito, L.M. and Nunes, L.J., 2023. Soil carbon sequestration in the context of climate change mitigation: A review. *Soil Sys.* 7(3):64.
- 31) Sanaullah, M., E. Blagodatskaya, A. Chabbi, C. Rumpel and Y. Kuzyakov. 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. *Appl. Soil Ecol.* 48:38-44.
- 32) Sarkar, S., Skalicky, M., Hossain, A., Brestic, M., Saha, S., Garai, S., Ray, K. and Brahmachari, K., 2020. Management of crop residues for improving input use efficiency and agricultural sustainability. *Sustainability*, 12(23):9808.
- 33) Sharma, S., H. Thind, H. Sidhu, M. Jat and C. Parihar. 2019. Effects of crop residue retention on soil carbon pools after 6 years of rice–wheat cropping system. *Environ. Earth. Sci.* 78:1-14.
- 34) Steponavičienė, V., Žiūraitis, G., Rudinskienė, A., Jackevičienė, K. and Bogužas, V., 2024. Long-Term Effects of Different Tillage Systems and Their Impact on Soil Properties and Crop Yields. *Agronomy*, 14(4), p.870.
- 35) Telles, T.S., Melo, T.R.D., Righetto, A.J., Didoné, E.J. and Barbosa, G.M.D.C., 2022. Soil management practices adopted by farmers and how they perceive conservation agriculture. *Revista Brasileira de Ciência do Solo*, 46:0210151.
- 36) ur Rehman, S., Ijaz, S.S., Raza, M.A., Din, A.M.U., Khan, K.S., Fatima, S., Raza, T., Mehmood, S., Saeed, A. and Ansar, M., 2023. Soil organic carbon sequestration and modeling under conservation tillage and cropping systems in a rainfed agriculture. *EJA.* 147:126840.
- 37) Vance, E.D., P.C. Brookes and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass c. *Soil Bio. Biochem.* 19:703-707.
- 38) Veni, V.G., Srinivasarao, C., Reddy, K.S., Sharma, K.L. and Rai, A., 2020. Soil health and climate change. In *Climate change and soil interactions*.751-767. Elsevier.
- 39) Walkley, A. and I.A. Black. 1934. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* 37:29-38.
- 40) Wang, X., X. Li, Y. Hu, J. Lv, J. Sun, Z. Li and Z. Wu. 2010. Effect of temperature and moisture on soil organic carbon mineralization of predominantly permafrost peatland in the great hing'an mountains, northeastern China. *J. Environ. Sci.* 22:1057-1066.
- 41) Xiao, D., Y. Ye, S. Xiao, W. Zhang, X. He and K. Wang. 2019. Effects of tillage on co2 fluxes in a typical karst calcareous soil. *Geoderma.* 337:191-201.