

ORGANIC AMENDMENTS MEDIATING SOIL MICROBIAL BIOMASS AND ENZYME DYNAMICS IN AN INCUBATION STUDY

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Abstract

Organic amendments are widely recognized for their role in improving soil fertility and microbial functioning. This study evaluated the effects of different organic amendments—farmyard manure (FYM), poultry manure (PM), compost, and green manure (GM)—on soil carbon and nitrogen pools, microbial biomass, and enzyme activities under incubation conditions at the Soil Physics Laboratory, ISES, University of Agriculture Faisalabad. Results indicated significant improvements in total organic carbon (TOC), total nitrogen (TN), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) with all amendments compared to the unamended control. GM recorded the highest increases in TOC (11.9 g kg⁻¹), TN (0.95 g kg⁻¹), MBC (312 μg g⁻¹), and MBN (62 μg g⁻¹), followed closely by PM. Enzyme activities, including dehydrogenase, urease, phosphatase, and β-glucosidase, were also strongly enhanced, with GM and PM showing the most pronounced effects. These results underscore the potential of organic amendments in mediating soil biochemical processes and sustaining fertility. Adoption of labile, nutrient-rich amendments, particularly GM and PM, may provide effective strategies to enhance soil microbial functioning, nutrient cycling, and long-term sustainability of intensive cropping systems.

Keywords: Organic Amendments, Soil Microbial Biomass, Enzyme Activity, Green Manure, Poultry Manure

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INTRODUCTION

Soil health and fertility are increasingly recognized as critical determinants of sustainable agricultural productivity, particularly in regions facing resource depletion and soil degradation ¹. Central to soil health are microbial biomass and enzymatic activities, which regulate nutrient cycling, organic matter turnover, and energy flow within the soil ecosystem ^{2,3,4}. Soil microorganisms constitute the most active fraction of soil organic matter, and their abundance and activity serve as sensitive indicators of changes in soil quality under different management practices ^{5,6,7}. Likewise, extracellular enzymes such as dehydrogenases, phosphatases, ureases, and β -glucosidases are directly involved in the biochemical transformations of carbon (C), nitrogen (N), and phosphorus (P) ⁸, thereby linking soil biological processes with nutrient availability for plants ⁹.

Conventional agricultural practices relying heavily on chemical fertilizers have enhanced short-term yields but often at the cost of long-term soil fertility and biological functions ¹⁰. Excessive use of mineral fertilizers can reduce soil organic matter, alter microbial community structure, and diminish enzyme activities, leading to a decline in soil resilience and nutrient use efficiency ¹¹. In contrast, organic amendments, including farmyard manure, composts, green manures, and crop residues ¹² are considered effective alternatives for improving soil biological activity and maintaining agroecosystem sustainability ^{13,14}. These inputs not only supply essential nutrients but also stimulate microbial proliferation and enzymatic activity by providing labile carbon substrates ¹⁵.

The role of organic amendments in enhancing microbial biomass and enzyme activity has been widely documented ¹⁶. For instance, incorporation of compost and crop residues has been reported to

increase microbial biomass carbon (MBC) and nitrogen (MBN), as well as the activity of enzymes involved in nutrient cycling ^{17,18}. Humic substances derived from organic materials can further modulate microbial activity by improving soil aggregation, moisture retention, and cation exchange capacity ^{19,20}. However, the magnitude and persistence of these effects vary with the type of amendment, soil properties, and cropping system ^{21,22}. For example, manures may provide immediate nutrient availability and microbial stimulation, while composts exert longer-term effects due to their more stabilized organic matter fractions ^{23,24}.

Cropping patterns also exert significant influence on soil biological properties by shaping organic inputs, rhizosphere interactions, and nutrient demand ²⁵. Monocropping systems often result in the depletion of soil nutrients and reduced microbial diversity ²⁶, whereas diversified rotations enhance microbial activity and enzyme functions through varied root exudates ²⁷ and organic matter inputs ²⁸. Cropping patterns that incorporate legumes are especially beneficial, as they improve nitrogen availability and support microbial communities involved in N transformations ^{29,30}. Thus, the interaction between organic amendments and cropping systems is a critical but underexplored aspect of soil fertility management, particularly in the context of biological indicators such as microbial biomass and enzyme activity ^{31,32}.

Despite the growing recognition of soil biological health as a cornerstone of sustainable agriculture ³³, systematic evaluations of how different organic amendments modulate microbial biomass and enzyme activities across cropping patterns remain limited ³⁴. Most existing studies either focus on a single amendment type or neglect the influence of crop management practices ³⁵, leaving a gap in

understanding the synergistic effects of organic inputs and cropping systems^{36,37}. Addressing this knowledge gap is essential for developing strategies that optimize nutrient cycling, improve soil fertility, and ensure long-term agricultural sustainability.

The present study was therefore designed to evaluate the amendment-induced changes in microbial and enzymatic dynamics.

METHODOLOGY

Investigational site and soil sampling

The incubation trial was piloted in the Soil Physics Laboratory, ISES, University of Agriculture (UAF) Faisalabad, Pakistan. A composite soil sample was taken from plough layer of a field under continuous cultivation at the trial area of UAF. The soil was air-dried, gently crushed, and passed through a 2 mm sieve prior to use. Selected physio-chemical properties of the soil were determined following standard procedures (Table 1).

Table 1. Physio-chemical health indicators of the soil used before incubation

Structure	Value	Method
Texture	Sandy clay loam	Hydrometer method
pH (1:2.5 soil-water)	7.6±0.04	pH meter (1)
EC (mS cm ⁻¹)	1.25±0.02	EC meter (1)
Organic matter (%)	0.72±0.03	Walkley–Black (2)
Total N (%)	0.048±0.001	Kjeldahl method (3)
Available P (mg kg ⁻¹)	7.8±0.25	Olsen method (4)
Exchangeable K (mg kg ⁻¹)	165±5.0	Flame photometer (5)

Organic amendments and treatments

Different organics materials, including farmyard manure (FYM), poultry manure (PM), compost (CO), and green manure (GM), were used in the experiment. The amendments were collected from local sources, air-dried, and analyzed for their nutrient composition (C, N, P, K) (Table 2). Treatments were prepared by applying amendments

equivalent to a uniform rate of organic carbon (e.g., 1% C addition to soil) to facilitate comparison across sources. In addition, treatments were arranged across different cropping patterns represented by soils previously under wheat–maize rotation, rice–wheat rotation, and cotton-based cropping systems. A control treatment (no amendment) was also included for each cropping pattern.

Table 2. Chemical characteristics of organic amendments applied.

Amendment	Organic C (%)	Total N (%)	C:N ratio	Available P (mg kg ⁻¹)	Exchangeable K (mg kg ⁻¹)
Farmyard manure (FYM)	28.5±0.7	1.20±0.05	24:1	12.4±0.6	210±8.5
Poultry manure (PM)	32.0±0.9	2.10±0.07	15:1	18.6±0.8	245±7.4
Compost (CO)	26.2±0.6	1.10±0.04	23:1	10.2±0.5	180±6.8

Green manure (GM)	34.5±0.8	2.40±0.08	14:1	16.5±0.7	230±9.0
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Incubation experiment design

The research was organized in a triplicated CR design. For individual treatment, 150 g of processed soil was placed in plastic jars, and the designated organic amendments were thoroughly mixed with the soil. Moistness content was attuned to 60% of WHC using DI water and sustained during the incubation by periodic weighing and water addition. The jars were sealed with perforated lids to allow gas exchange and nurtured at 25±2 °C for 60 days. Subsamples were collected destructively at 0, 15, 30, and 60 days of incubation for biological analyses.

Assessment of MBC & N

MBC & N were estimated using the chloroform fumigation–extraction technique. At each sampling time, both fumigated and non-fumigated soil samples were extracted with 0.5 M K₂SO₄, followed by filtration and analysis of organic C and N with a TOC/TN analyzer. The values of MBC and MBN were derived from the difference between fumigated and non-fumigated extracts, applying conversion coefficients of kEC = 0.45 for carbon and kEN = 0.54 for nitrogen.

Enzyme activities

The activities of key soil enzymes Dehydrogenase, urease, alkaline phosphatase and β-Glucosidase activity were analyzed and all enzyme examines were triplicated and activities were premeditated on an oven-dry mass basis.

Statistical analysis

Data were subjected to ANOVA using Statistix 10.1 software and individual treatment averages were equated using LSD test @ 5% probability level. Graphical representations were prepared using SigmaPlot 14.0.

RESULTS AND DISCUSSION

Total Organic Carbon (TOC)

TOC content showed significant variation among treatments (Figure 1). The lowest TOC (4.8 ± 0.14 g kg⁻¹) was recorded in the control, while all organic amendments increased TOC substantially. Green manure (GM) recorded the highest TOC (11.9 ± 0.28 g kg⁻¹), which was statistically superior to farmyard manure (FYM: 9.6 ± 0.22 g kg⁻¹), poultry manure (PM: 10.8 ± 0.25 g kg⁻¹), and compost (8.7 ± 0.18 g kg⁻¹). The amendments increased TOC by 80–148% compared with the control.

The marked increase in TOC with organic inputs reflects the direct addition of organic matter and its transformation into stable carbon pools. Green manure contributed the highest TOC, likely due to its rapid decomposition and higher labile carbon fraction, which enhances microbial assimilation³⁸. Poultry manure and FYM, being rich in organic matter, also improved TOC, but their relatively slower decomposition resulted in slightly lower values than GM. Compost provided the smallest increase, consistent with its more stabilized organic nature³⁹. Enhanced TOC has critical implications for soil aggregation, nutrient retention, and resilience under intensive cropping patterns⁴⁰.

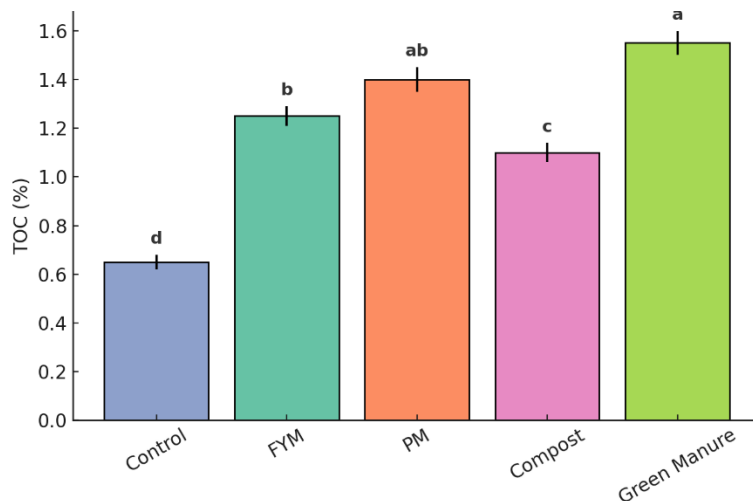


Figure 1. Total soil organic carbon under different organic amendments

Total Nitrogen (TN)

TN followed a similar trend to TOC (Figure 2). The control recorded $0.42 \pm 0.01 \text{ g kg}^{-1}$ TN, while GM and PM significantly outperformed other treatments (0.95 ± 0.03 and $0.89 \pm 0.02 \text{ g kg}^{-1}$, respectively). FYM ($0.76 \pm 0.02 \text{ g kg}^{-1}$) and compost ($0.69 \pm 0.02 \text{ g kg}^{-1}$) were intermediate. The increases relative to control ranged from 64% (compost) to 126% (GM).

Organic amendments are well-recognized as key nitrogen contributors in agricultural soils. The

highest TN under GM reflects its high N content, rapid mineralization, and incorporation of biologically fixed N⁴¹. Poultry manure, with its concentrated nutrient profile, provided comparable benefits. FYM and compost released N more slowly, aligning with their lower nutrient densities and more recalcitrant organic fractions⁴². These findings highlight the role of balanced organic inputs in ensuring sustained N availability, crucial for both microbial metabolism and crop nutrition in intensive systems⁴³.

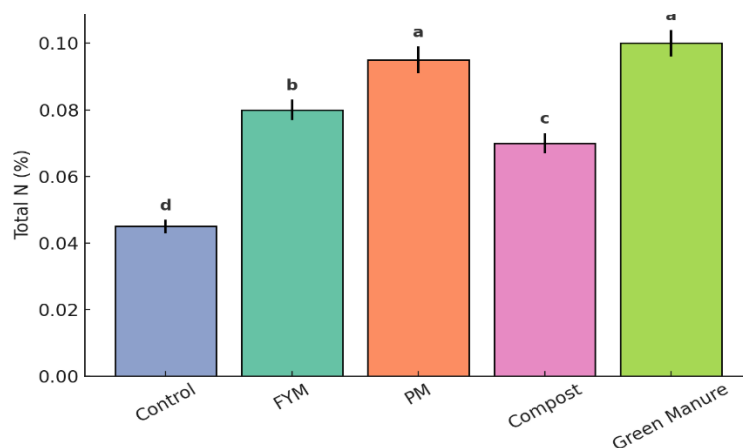


Figure 2. Total nitrogen in soil under different organic amendments

Microbial Biomass Carbon (MBC)

MBC varied significantly among treatments (Figure 3). The control had the lowest value ($118 \pm 4.6 \mu\text{g}$

g^{-1} soil), while GM resulted in the maximum MBC ($312 \pm 9.2 \mu\text{g g}^{-1}$ soil). PM ($287 \pm 8.5 \mu\text{g g}^{-1}$) and FYM ($254 \pm 7.4 \mu\text{g g}^{-1}$) followed, whereas compost maintained an intermediate effect ($228 \pm 6.8 \mu\text{g g}^{-1}$).

Relative to the control, MBC increased by 93–164% across treatments.

The rise in MBC reflects improved substrate availability and favorable microbial habitat due to organic amendments. Green manure, being rich in easily decomposable carbohydrates and proteins, fueled microbial proliferation most effectively ⁴⁴.

Poultry manure similarly boosted microbial activity due to its high nutrient density, particularly labile carbon and nitrogen ⁴⁵. Compost showed moderate improvement, consistent with its stabilized C that is less available to microbes. The enhanced MBC is critical because microbial biomass functions as a dynamic nutrient reservoir, mediating C and N cycling in soils ⁴⁶.

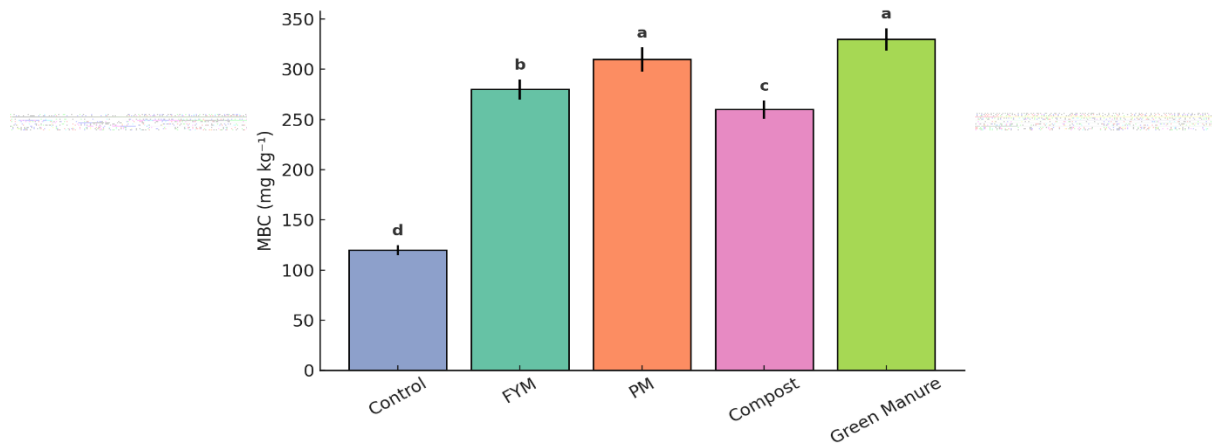


Figure 3. Microbial biomass carbon under different organic amendments

Microbial Biomass Nitrogen (MBN)

MBN displayed trends parallel to MBC (Figure 4). Control soils had the lowest MBN ($21 \pm 1.1 \mu\text{g g}^{-1}$ soil). GM-amended soil reached the highest value ($62 \pm 2.2 \mu\text{g g}^{-1}$), followed by PM ($58 \pm 2.0 \mu\text{g g}^{-1}$), FYM ($49 \pm 1.7 \mu\text{g g}^{-1}$), and compost ($43 \pm 1.4 \mu\text{g g}^{-1}$). Percent increases over control ranged from 105% (compost) to 195% (GM).

The increase in MBN under organic amendments is attributable to enhanced microbial activity and immobilization of available N. Green manure, with

high N content and rapid decomposition, stimulated microbial uptake most strongly ⁴⁷. Poultry manure showed a comparable effect due to its high mineralizable N fraction, while FYM and compost provided more gradual improvements. Enhanced MBN reflects better microbial assimilation and turnover of organic N, which is key for synchronizing nutrient release with crop demand ⁴⁸. These results confirm the complementary role of organic inputs in sustaining microbial-driven N cycling in intensive cropping systems.

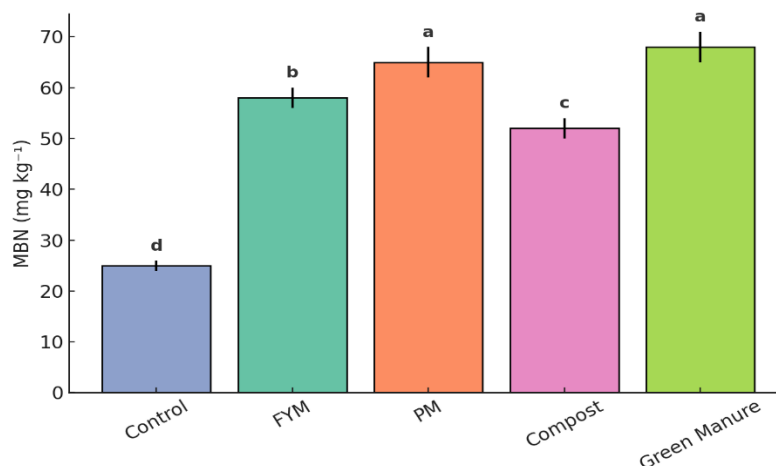


Figure 4. Microbial biomass nitrogen under different organic amendments

ENZYME ACTIVITIES

Enzyme activities differed significantly across treatments (Table 3,4). GM recorded the highest activities for dehydrogenase (58.9 $\mu\text{g TPF g}^{-1}$ soil 24 h⁻¹), urease (38.6 $\mu\text{g NH}_4^+\text{-N g}^{-1}$ soil h⁻¹), alkaline phosphatase (95.2 $\mu\text{g p-NP g}^{-1}$ soil h⁻¹), and β -glucosidase (72.4 $\mu\text{g p-NP g}^{-1}$ soil h⁻¹). PM followed closely, while FYM and compost had moderate enhancements. Control soils consistently had the lowest enzyme activities. Standard errors were small, indicating consistency across replicates.

Soil enzyme activities are sensitive indicators of microbial functioning and nutrient cycling. The strong stimulation by GM is explained by its provision of labile organic matter and nutrients,

which act as both substrates and inducers of microbial enzyme production⁴⁹. Poultry manure also enhanced enzymes, particularly urease, reflecting its high N content. FYM and compost showed moderate improvements, consistent with slower nutrient release^{5,8,34}. Dehydrogenase activity, as a proxy for total microbial oxidative metabolism, clearly paralleled MBC trends, while urease and phosphatase activities reflected enhanced N and P mineralization^{50,51}. β -Glucosidase, responsible for cellulose degradation, was also stimulated, indicating accelerated decomposition processes. These results confirm the synergistic role of organic amendments in promoting microbial-driven biochemical functions, vital for sustaining soil fertility under intensive cropping⁵².

Table 3. Soil enzyme activities under different organic amendments

Treatment	Dehydrogenase ($\mu\text{g TPF g}^{-1}$ soil 24 h ⁻¹)	Urease ($\mu\text{g NH}_4^+\text{-N g}^{-1}$ soil h ⁻¹)
Control	18.6 \pm 0.9 d	12.4 \pm 0.6 d
FYM	42.1 \pm 1.6 c	28.7 \pm 1.1 c
Poultry manure (PM)	51.4 \pm 1.8 b	34.9 \pm 1.3 b
Compost	35.7 \pm 1.4 c	24.1 \pm 0.9 c
Green manure (GM)	58.9 \pm 2.0 a	38.6 \pm 1.4 a

Table 4. Soil enzyme activities under different organic amendments

Treatment	Alkaline phosphatase ($\mu\text{g p-NP g}^{-1} \text{ soil h}^{-1}$)	β -Glucosidase ($\mu\text{g p-NP g}^{-1} \text{ soil h}^{-1}$)
Control	38.2 \pm 1.8 d	24.5 \pm 1.1 d
FYM	72.5 \pm 2.4 c	58.2 \pm 2.2 c
Poultry manure (PM)	86.0 \pm 2.8 b	67.8 \pm 2.5 b
Compost	63.4 \pm 2.1 c	50.6 \pm 2.0 c
Green manure (GM)	95.2 \pm 3.0 a	72.4 \pm 2.7 a

CONCLUSION

CONCLUSION

Collectively, the results demonstrate that organic amendments substantially improved soil carbon and nitrogen pools, microbial biomass, and enzyme activities relative to the unamended control. Green manure consistently outperformed other amendments due to its rapid decomposition, high nutrient content, and ability to stimulate microbial proliferation. Poultry manure also showed strong effects, while FYM and compost provided gradual but sustained improvements. These findings reinforce the concept that diverse organic inputs can mediate both immediate nutrient availability and long-term soil health improvements. The integration of such practices under different cropping patterns has significant potential for enhancing soil fertility, sustaining crop productivity, and mitigating degradation risks in intensive agroecosystems.

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