Soil Enzyme Activity and Microbial Biomass Carbon as Indicators of Sustainable Crop Production

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ABSTRACT
Background: Soil biological properties respond rapidly to management and provide early indication of changes in soil fertility long before physicochemical parameters shift measurably.
Objective: This study evaluated soil enzyme activities and microbial biomass carbon (MBC) as indicators of soil health under contrasting nutrient management systems and examined their relationship with crop productivity.
Methods: A field experiment was conducted in a randomized complete block design with six treatments—control, chemical fertilizer, farmyard manure, compost, biofertilizer, and integrated snutrient management (INM)—replicated thrice. Soil samples (0–15 cm) were analyzed for physicochemical properties, MBC (chloroform fumigation-extraction), and dehydrogenase, urease, β-glucosidase, acid phosphatase, alkaline phosphatase, and catalase activities. Crop growth, yield, and nutrient uptake were recorded, and data were subjected to ANOVA, correlation, and principal component analysis.
Results: INM and compost treatments recorded the highest MBC and enzyme activities, significantly ($P < 0.05$) exceeding the control and chemical fertilizer treatments. MBC correlated positively with organic carbon ($r = 0.86$) and grain yield ($r = 0.79$). Dehydrogenase and β-glucosidase showed the strongest associations with nutrient uptake and yield.
Conclusion: Integrated and organic-based nutrient management enhanced soil biological activity and crop productivity simultaneously, confirming MBC and enzyme assays as sensitive, cost-effective indicators for monitoring soil health in sustainable cropping systems.

Keywords: Soil health; Microbial biomass carbon (MBC); Soil enzyme activities; Integrated nutrient management (INM)

1. Introduction
Soil health underpins the long-term sustainability of agricultural production systems, yet conventional fertility assessment based solely on physicochemical properties often fails to detect early degradation or improvement in functional soil quality [1]. Biological indicators—particularly microbial biomass carbon (MBC) and soil enzyme activities—respond more rapidly to management-induced changes than total organic carbon or nutrient pools, making them valuable early-warning tools [2, 3].
MBC represents the living, metabolically active fraction of soil organic matter that mediates nutrient transformation, immobilization, and mineralization [4]. Soil enzymes, largely of microbial origin, catalyze critical biochemical reactions in carbon, nitrogen, and phosphorus cycling; dehydrogenase reflects overall microbial oxidative activity, urease and phosphatases govern nitrogen and phosphorus availability, β-glucosidase drives cellulose degradation, and catalase indicates aerobic microbial metabolism [5, 6]. Collectively, these biological attributes integrate the effects of substrate quality, moisture, aeration, and management history, offering a holistic measure of soil functional capacity [7].
Agricultural management practices—tillage intensity, residue retention, and nutrient source—strongly influence microbial communities and their enzymatic output [8]. Excessive reliance on chemical fertilizers, without organic inputs, can suppress microbial biomass and enzyme synthesis over time, whereas organic amendments and integrated nutrient management (INM) sustain microbial diversity and activity [9]. Despite growing recognition of these relationships, few field studies simultaneously quantify MBC, a full enzyme suite, and crop performance across a nutrient management gradient.
This study therefore aimed to (i) evaluate the effect of different nutrient management systems on soil MBC and enzyme activities, (ii) determine their relationships with soil physicochemical properties, and (iii) assess their utility as predictive indicators of crop productivity, thereby providing a scientific basis for selecting sustainable soil management strategies.

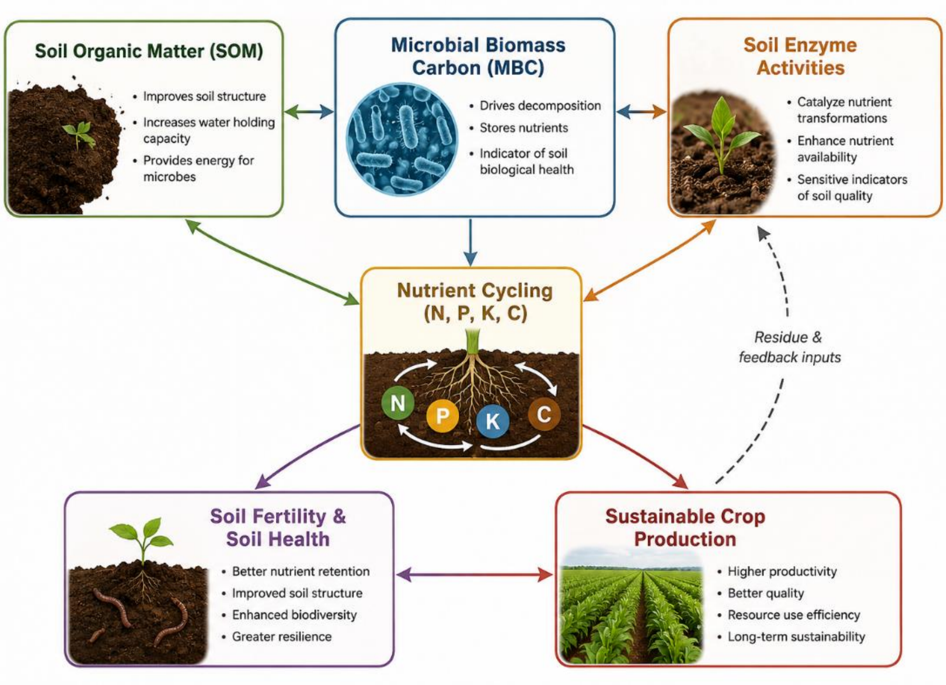

Fig 1: Conceptual linkages among soil organic matter, microbial biomass carbon, soil enzyme activities, nutrient cycling, and sustainable crop production

2. Literature Review

Numerous studies have established MBC as a sensitive early indicator of soil organic matter dynamics, typically responding to management changes within one to two cropping cycles, well before bulk organic carbon shows detectable differences [2, 10]. Comparative assessments of organic, inorganic, and integrated nutrient sources consistently report higher MBC and dehydrogenase activity under organic and integrated systems relative to sole chemical fertilization [9, 11]. Soil enzyme activities have been widely used as diagnostic tools for soil quality assessment. Dehydrogenase, urease, and phosphatase activities generally increase with organic carbon input and decline under intensive tillage or monocropping without residue return [5, 12]. β-Glucosidase, being closely tied to labile carbon availability, has been proposed as a particularly responsive indicator of short-term carbon dynamics [6, 13].

Conservation agriculture practices—reduced tillage, residue retention, and crop diversification—have been shown to enhance soil aggregate stability, microbial biomass, and enzymatic function by improving habitat continuity and substrate supply for soil biota [8, 14]. Similarly, soil carbon cycling research emphasizes the role of microbial necromass and enzyme-mediated decomposition in stabilizing organic matter within aggregates [15].

Studies on soil microbial ecology further indicate that fungal-to-bacterial ratios and microbial community composition shift markedly with long-term organic input, favoring decomposition pathways that promote aggregate stabilization and more efficient carbon turnover [17, 18]. Nutrient management history also interacts with cropping system diversity; rotations incorporating legumes have been reported to elevate MBC and urease activity through enhanced biological nitrogen fixation and root exudation [19, 20].

Despite this substantial body of work, gaps remain regarding the simultaneous, multi-enzyme characterization of soil biological quality alongside crop yield response under realistic, multi-treatment field conditions, particularly in semi-arid cropping systems where seasonal moisture fluctuation strongly modulates microbial activity [3, 16]. Moreover, few studies integrate correlation and multivariate approaches such as PCA to disentangle the relative contribution of individual enzymes versus MBC to overall soil functional quality. This study addresses these gaps through an integrated field-based assessment combining biological, physicochemical, and yield parameters across a realistic nutrient management gradient.

3. Materials and Methods

3.1. Experimental Site

The field experiment was conducted over two consecutive cropping seasons at a research farm situated in a semi-arid agro-climatic zone (mean annual rainfall 750 mm, mean temperature 24°C). The soil was a sandy clay loam (*Typic Haplustept*), slightly alkaline, under a cereal–legume rotation cropping system.

3.2. Experimental Design

A randomized complete block design (RCBD) with three replications was used, comprising six treatments: T1—control (no nutrient input); T2—recommended chemical fertilizer (NPK); T3—farmyard manure (FYM, 10 t ha^{-1}); T4—compost (7.5 t ha^{-1}); T5—biofertilizer (*Azotobacter* + PSB consortium); and T6—integrated nutrient management (50% NPK + FYM + biofertilizer) (Table 1).

Table 1: Experimental treatments and soil management practices

Treatment	Description	Nutrient Source
T1	Control	No nutrient input
T2	Chemical fertilizer	Recommended NPK (100%)
T3	Farmyard manure (FYM)	FYM @ 10 t ha^{-1}
T4	Compost	Compost @ 7.5 t ha^{-1}
T5	Biofertilizer	*Azotobacter* + PSB consortium
T6	Integrated Nutrient Management (INM)	50% NPK + FYM + biofertilizer

3.3. Soil Sampling
Composite soil samples were collected from 0–15 cm depth at crop harvest from five random points per plot, pooled, sieved (2 mm), and stored at 4°C for biological analyses and air-dried for physicochemical analyses.

3.4. Soil Physicochemical Analysis
Soil pH and electrical conductivity were measured in 1:2.5 soil–water suspension; organic carbon by the Walkley–Black method; available nitrogen by alkaline permanganate method; available phosphorus by Olsen's method; available potassium by flame photometry; and soil moisture gravimetrically.

3.5. Soil Biological Analysis
MBC was determined by the chloroform fumigation-extraction method using a Kec factor of 0.38. Enzyme activities were assayed as follows: dehydrogenase (triphenyl tetrazolium chloride reduction), urease (urea hydrolysis to ammonium), β-glucosidase and acid/alkaline phosphatase (p-nitrophenyl substrate colorimetry), and catalase (titrimetric hydrogen peroxide decomposition).

3.6. Crop Measurements
Plant height, above-ground biomass, grain yield, harvest index, and nitrogen–phosphorus–potassium uptake were recorded at physiological maturity.

3.7. Statistical Analysis
Data were analyzed by two-way ANOVA; treatment means were separated using Tukey's HSD test at $P < 0.05$. Pearson correlation and principal component analysis (PCA) were performed to examine relationships among biological and yield parameters using R software (v4.3).

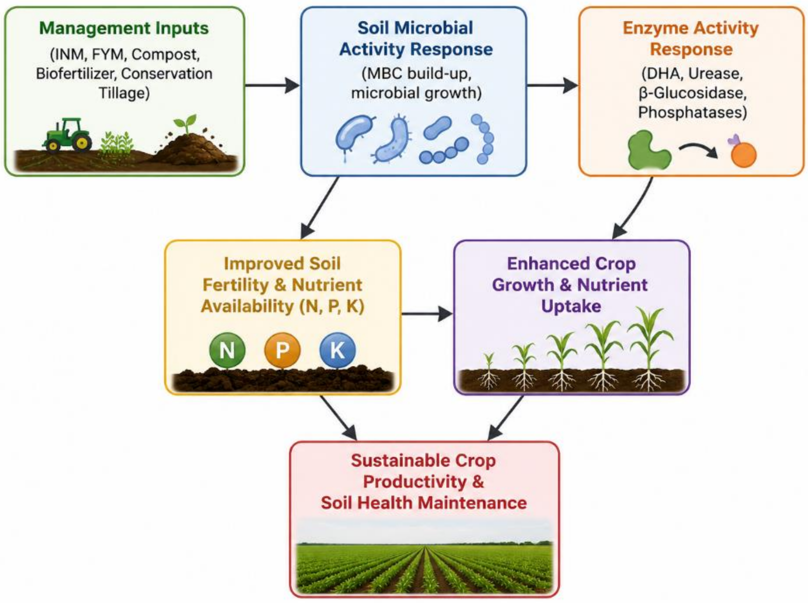

Fig 2: Workflow illustrating the influence of integrated nutrient management, organic amendments, and conservation practices on soil microbial activity, enzyme responses, soil fertility, and crop productivity

4. Results
4.1. Soil Physicochemical Properties
Organic carbon, available N, P, K, and soil moisture were significantly higher under T6 (INM) and T4 (compost) than under T1 (control) and T2 (chemical fertilizer) (Table 2). Soil pH remained largely unaffected across treatments, while electrical conductivity showed a marginal, non-significant decline under organic treatments.

Table 2: Soil physicochemical properties and microbial biomass carbon (mean ± SE)

Treatment	pH	OC (%)	Avail. N (kg/ha)	Avail. P (kg/ha)	Avail. K (kg/ha)	MBC (μg/g)
T1	7.6±0.05	0.42±0.02	178±5	12.4±0.6	142±4	198±9a
T2	7.5±0.04	0.48±0.02	205±6	15.8±0.7	158±5	241±10b
T3	7.4±0.05	0.61±0.03	232±7	19.2±0.8	176±5	322±11c
T4	7.4±0.04	0.64±0.03	241±6	20.5±0.7	182±6	351±12cd
T5	7.5±0.05	0.55±0.02	219±6	17.6±0.6	168±5	289±10bc
T6	7.4±0.03	0.68±0.02	258±7	22.9±0.8	191±6	386±13d

Values followed by different letters differ significantly ($P < 0.05$, Tukey's HSD)

4.2. Microbial Biomass Carbon

MBC ranged from 198 µg g^{-1} soil in the control to 386 µg g^{-1} soil under INM, representing a 95% increase (Table 2). Compost and FYM treatments also showed substantial MBC enhancement over chemical fertilizer alone ($P < 0.05$). MBC correlated strongly with organic carbon ($r = 0.86$, $P < 0.01$), confirming its dependence on substrate carbon supply.

4.3. Soil Enzyme Activities

All six enzymes showed significantly higher activity under INM and compost treatments relative to control and chemical fertilizer alone (Table 3). Dehydrogenase activity increased by approximately 120% under INM over control, while β-glucosidase and alkaline phosphatase showed 85–100% increases. Catalase activity, though responsive, showed comparatively smaller treatment differences, indicating lower sensitivity to nutrient source.

Table 3: Activities of major soil enzymes under different treatments (mean ± SE)

Treatment	Dehydrogenase (µg TPF/g/24h)	Urease (µg NH4-N/g/h)	β-Glucosidase (µg PNP/g/h)	Acid Phosphatase (µg PNP/g/h)	Alkaline Phosphatase (µg PNP/g/h)	Catalase (mL O2/g/min)
T1	18.4±1.1a	24.6±1.3a	62.1±3.0a	48.3±2.4a	51.7±2.6a	1.42±0.08a
T2	24.7±1.3b	31.2±1.4b	78.5±3.2b	58.9±2.6b	62.4±2.8b	1.58±0.09ab
T3	33.6±1.5c	39.8±1.6c	98.7±3.6c	71.5±2.9c	76.2±3.1c	1.71±0.10bc
T4	36.2±1.6cd	42.1±1.7cd	105.3±3.8cd	75.8±3.0cd	82.6±3.2cd	1.79±0.10c
T5	29.4±1.4bc	35.7±1.5bc	88.9±3.4bc	65.2±2.7bc	69.8±2.9bc	1.65±0.09bc
T6	40.5±1.7d	46.3±1.8d	114.8±4.0d	81.6±3.1d	89.3±3.3d	1.86±0.11c

Values followed by different letters differ significantly (P < 0.05, Tukey's HSD). TPF = triphenyl formazan; PNP = p-nitrophenol

4.4. Crop Performance

Grain yield was highest under INM (4.82 t ha^{-1}), followed by compost (4.51 t ha^{-1}), both significantly exceeding chemical fertilizer (3.96 t ha^{-1}) and control (2.74 t ha^{-1}). Nutrient uptake followed a similar pattern, with nitrogen uptake nearly doubling under INM relative to control.

4.5. Correlation and Principal Component Analysis

MBC and dehydrogenase activity showed the strongest positive correlations with grain yield ($r = 0.79$ and $r = 0.81$, respectively, $P < 0.01$) (Table 4). PCA extracted two principal components explaining 78% of total variance; PC1 was dominated by MBC, dehydrogenase, and β-glucosidase, clearly separating organic/integrated treatments from control and chemical fertilizer along the biological-activity axis.

Table 4: Correlation among microbial biomass carbon, soil enzyme activities, soil properties, and crop yield

Parameter	Organic Carbon	MBC	Dehydrogenase	β-Glucosidase	Grain Yield
Organic Carbon	1.00	0.86**	0.80**	0.77**	0.74**
MBC	—	1.00	0.88**	0.83**	0.79**
Dehydrogenase	—	—	1.00	0.85**	0.81**
β-Glucosidase	—	—	—	1.00	0.76**
Grain Yield	—	—	—	—	1.00

**Correlation significant at P < 0.01 (two-tailed).

5. Discussion

The consistent enhancement of MBC and enzyme activities under compost and INM treatments reflects the greater substrate availability and habitat quality provided by organic carbon inputs, which sustain larger, more metabolically active microbial communities [4, 9]. The strong correlation between MBC and organic carbon corroborates earlier findings that microbial biomass functions as a labile, readily mineralizable carbon pool sensitive to short-term management change [2, 10].

Elevated dehydrogenase and phosphatase activities under organic-based treatments indicate improved oxidative metabolism and phosphorus mineralization capacity, directly supporting nutrient availability for crop uptake [5, 12]. The comparatively modest response of catalase aligns with its broader distribution across both biotic and abiotic soil components, limiting its diagnostic specificity relative to dehydrogenase or β-glucosidase [6].

The positive association between biological indicators and grain yield reinforces the concept that soil microbial functions are not merely passive correlates of organic input but active drivers of nutrient cycling that translate into measurable productivity gains [3, 13]. These findings are consistent with reports from conservation agriculture systems, where residue retention and reduced disturbance similarly enhanced enzymatic function and yield stability [8, 14].

Practically, these results support the incorporation of MBC and a concise enzyme panel (dehydrogenase, β-glucosidase, phosphatase) into routine soil health monitoring frameworks, offering rapid, biologically meaningful feedback on management effectiveness that is considerably less costly and time-intensive than full microbial community profiling. The PCA-based separation of treatments along a distinct biological-activity axis further suggests that a small subset of biological indicators can effectively discriminate management-induced soil quality differences without requiring the full physicochemical suite, which has practical value for large-scale soil monitoring programs with limited analytical capacity.

However, this study was limited to two seasons at a single site; enzyme responses can vary with seasonal moisture and temperature fluctuations not fully captured here [16], and the relatively short duration may understate the cumulative, long-term benefits of organic carbon build-up under INM and compost treatments. Additionally, catalase's comparatively weak treatment discrimination suggests that not all commonly measured enzymes carry equal diagnostic value, warranting more selective indicator panels in future protocols. Future multi-location, multi-year studies incorporating microbial community sequencing alongside enzymatic and yield data would strengthen the mechanistic understanding of biology–productivity linkages, refine threshold values for soil biological health, and improve the predictive power of soil biological indicators across diverse agro-ecological zones.

6. Conclusion

This study demonstrates that microbial biomass carbon and soil enzyme activities—particularly dehydrogenase, β-glucosidase, and phosphatases—are sensitive, reliable indicators of soil biological quality that respond rapidly to nutrient management practices. Integrated nutrient management and compost application consistently enhanced soil biological activity, nutrient availability, and grain yield compared with chemical fertilization alone, underscoring the value of combining organic and inorganic nutrient sources. Routine monitoring of MBC and key enzyme activities can therefore serve as a practical, cost-effective tool for guiding sustainable soil and crop management decisions. Future research should extend these assessments across diverse agro-ecological zones and cropping seasons, integrating molecular microbial community analysis to further refine soil health indicator frameworks for sustainable agriculture.

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