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Effect of long-term organic fertilization on soil chemical, biological properties and labile organic carbon fractions under maize (*Zea mays* L.) cultivation

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Abstract

Organic fertilization has been known to improve soil health by accelerating microbial activity, which mainly regulated the formation and decomposition of labile organic carbon (LOC). The objectives of this study were to: (1) analyze the effects of different fertilization on soil microbial activity; β -glucosidase, dehydrogenase, phosphatase, urease and soil respiration (RES) and LOC; particulate organic carbon (POC), permanganate oxidizable carbon (POXC), and microbial biomass carbon (MBC), and (2) evaluate the potential of LOC pool as a soil health indicator through correlation analysis. Treatments included chemical fertilizer (NPK), cattle manure compost (COM), green manure crops (GM), and an unfertilized control (NF). Soil chemical properties and microbial activity were higher in organic resource treatments (COM, GM) than in NPK. The COM treatment showed the highest values for pH, total nitrogen (T-N), soil organic matter, available phosphorus, and exchangeable cations. Also, microbial enzyme activities (β -glucosidase, dehydrogenase, phosphatase, urease) and RES were higher in organic fertilization, compared to NPK. In particular, COM treatment increased urease activity and respiration by 35% and 60%, respectively, relative to NPK. Significant positive correlations were observed between soil microbial activity and the LOC fractions, in particular, POC showed the strongest correlation ($p < 0.01$). Particulate organic C could be suggested as a reliable indicator for soil health. Additionally, maize yield of the organic treatments was comparable to those of NPK, confirming organic fertilization could be an effective management for both soil health and crop yield.

Keywords Biological properties, Carbon fraction, Soil health, Organic farming

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Introduction

Organic farming is an agricultural system designed to enhance nutrient cycling and soil carbon sequestration by substituting synthetic chemicals with organic amendments. These practices are fundamental to improving soil health—defined as the soil’s sustained capacity to function as a vital living ecosystem that supports biological productivity and environmental quality [1]. By fostering a robust foundation for ecosystem services, organic soil management ensures stable crop yields and long-term agricultural sustainability [2].

Among the most commonly used organic resources, livestock manure compost and green manure supply organic matter, a principal energy source for soil microorganisms, and provide essential nutrients to crops through the mineralization of organic matter. In Korea, livestock manure compost is widely used due to its high content of macronutrients such as nitrogen, phosphorus, potassium, and enhancement of soil health by increasing soil carbon storage and promoting aggregate formation [3]. Hairy vetch, a widely cultivated green manure crop, fixes atmospheric nitrogen in the soil. Although its biomass production is not much, its high nitrogen content and low C: N ratio facilitates efficient nutrient supply [4, 5]. Furthermore, hairy vetch suppresses soil erosion and increases soil organic matter content, thereby contributing to overall soil health [6].

Soil management using organic resources influences the accumulation and decomposition of soil organic matter and catalyzes biochemical reactions in the soil, serving as a central component of the “crop–microbial activity–soil nutrient” [7]. Moreover, microbial activity plays a major role in regulating the soil carbon cycle by governing the formation and decomposition of labile organic carbon (LOC) pools, including particulate organic carbon (POC), permanganate-oxidizable carbon (POXC), and microbial biomass carbon (MBC), through diverse biochemical pathways [8]. The interaction between soil microorganisms and LOC pools is key determinant of soil ecosystem function and carbon sequestration. This is the reason why these are recognized as indicators for assessing soil health across diverse environmental contexts [9, 10]. However, limited studies have examined the organic fertilization impact on labile organic carbon (LOC) pools and microbial activity, as well as their interrelationship.

In this study, we focused on labile organic carbon (LOC) fractions and enzyme activities because they serve as sensitive indicators that respond more rapidly to organic management than total organic carbon, reflecting the dynamic nature of soil biological health. Therefore, the objectives of this study were: (1) to assess the effects of organic resource application on LOC dynamics and soil microbial activity, and (2) to explore the correlations between these factors to evaluate the potential of LOC as a reliable soil health indicator. In addition, soil chemical properties and crop productivity were investigated to determine whether organic fertilization could improve soil health and provide a sustainable alternative to conventional chemical fertilization.

Materials and methods

Site description and experimental design

This study was conducted in experimental field station in Wanju province, Korea. The long-term organic farming experimental plot was established in 2015 and has been maintained over 10 years. The field trial was arranged in a randomized complete block design with three replicates and comprised four nutrient management treatments: no fertilizer (NF), chemical fertilizer (NPK), cattle manure compost (COM), and green manure (GM). The cropping system consisted of annual maize (*Zea mays* L.) cultivation from mid-May to mid-August. In the GM treatment plots, hairy vetch was grown as a winter cover crop and incorporated annually before maize transplanting, while other plots (NF, NPK, and COM) remained fallow during the winter period. The standard fertilization rate ($\text{N-P}_2\text{O}_5\text{-K}_2\text{O} = 18.6\text{-}3.5\text{-}7.4 \text{ kg } 10\text{a}^{-1}$, Rural Development Administration) of inorganic fertilizers were applied for NPK. The cattle manure compost (COM) was a commercially available product (livestock manure 60–70%, sawdust 30–40%) that met the maturity standards of the Rural Development Administration (RDA). For the green manure (GM) treatment, hairy vetch (*Vicia villosa* R.) was sown in October ($10 \text{ kg } 10\text{a}^{-1}$), harvested at the flowering stage in May, and incorporated into the soil. Both amendments were applied based on an equivalent nitrogen input of $18.6 \text{ kg } 10\text{a}^{-1}$. The nutrient composition characteristics of both the cattle manure compost and hairy vetch used over the 10-year period are summarized in Table 1.

Soil sampling and chemical properties analysis

Soil samples were collected from the 0–15 cm depth and air-dried for at least seven days and passed through a 2 mm sieve. Soil pH, electrical conductivity (EC), available P_2O_5 (Av. P_2O_5), and exchangeable cations were determined according to the soil analysis manual of the Rural Development Administration [11]. Total carbon (T-C)

Table 1 Chemical properties of organic amendment used for this study

Treatment	T-C (%)	T-N (%)	P ₂ O ₅ (%)	K ₂ O (%)	C: N ratio
Hairy vetch	44.45	3.62	0.53	3.68	12.28
Compost	31.04	3.08	4.32	5.51	10.08

T-N, total nitrogen; T-C, total carbon

and total nitrogen (T-N) contents were measured using an elemental analyzer (Elementar, Vario Max, Germany).

Soil enzyme activity and respiration analysis

Soil microbial activity was assessed through a series of enzyme activity assays. The activity of β -glucosidase (GLU) was determined following the procedure of the previous research [12]. Briefly, 0.5 g of fresh soil was incubated with 0.1 ml toluene, 1.5 ml citric phosphate buffer (pH 4.9), 0.9 ml distilled water, and 0.6 ml p-nitrophenyl- β -D-glucopyranoside (PNG). The mixture was vortexed and incubated at 30 °C for 1 h. After incubation, 8 ml ethanol was added, shaken for 15 s, and filtered. Two milliliters of 2 M Tris solution were added to the filtrate to develop color, and absorbance was measured at 400 nm using a U/Vis spectrophotometer (SPECORD-200 PLUS, Analytik Jena, Germany).

Phosphatase (PHA) activity was analyzed according to [13]. For this assay, 0.5 g of fresh soil was incubated with 0.1 ml toluene, 0.6 ml 0.5 M Tris buffer, 1.8 ml distilled water, and 0.6 ml p-nitrophenyl phosphate (pNPP). The suspension was vortexed and incubated at 30 °C for 1 h. Following incubation, 8 ml ethanol was added, shaken for 15 s, and filtered. Two milliliters of 2 M Tris solution were added to the filtrate, and absorbance was measured at 400 nm using a UV/Vis (SPECORD-200 PLUS, Analytik Jena, Germany).

Dehydrogenase (DHA) activity was determined following [14]. A 20 g fresh soil sample was mixed with 0.2 g CaCO_3 , to which triphenyl tetrazolium chloride (TTC) solution and distilled water were added. The mixture was sealed and incubated at 37 °C for 24 h. After incubation, methanol was added, and the mixture was filtered through a funnel lined with defatted cotton, with additional methanol added until the red coloration disappeared. The filtrate was then brought to a final volume of 100 ml with methanol, and absorbance was recorded at 485 nm using a UV/Vis spectrophotometer (SPECORD-200 PLUS, Analytik Jena, Germany).

Urease (URE) activity was assayed as described by [15]. Briefly, 5 g of fresh soil was incubated with 2.5 ml of 79.9 mM urea solution at 37 °C for 2 h. After incubation, distilled water and KCl were added, and the mixture was shaken for 30 min before filtration. For the control, distilled water was used in place of the substrate solution. One milliliter of filtrate was diluted with 4 ml distilled water, followed by the addition of a reagent mixture containing 0.3 M NaOH, sodium salicylate solution, and distilled water, together with 39.1 mM dichloroisocyanurate solution. After color development by shaking for 30 min, absorbance was measured at 690 nm with a UV-Vis spectrophotometer (SPECORD-200 PLUS, Analytik Jena, Germany).

In addition to enzyme activities, soil respiration (RES) was measured as an indicator of microbial activity, following [16]. For this assay, 100 g of fresh soil was placed into a 300 ml bottle, with a vial containing 15 ml of 0.5 M NaOH fixed in the center of the bottle. The bottles were sealed and incubated at 25 °C for 72 h. After incubation, the residual NaOH was titrated with 1 M HCl using phenolphthalein as an indicator until the solution turned from purple to colorless, followed by titration with 0.2 M HCl using bromocresol green as an indicator until the color changed from blue to yellow. The amount of CO_2 evolved was calculated based on the volume of HCl consumed.

Soil labile organic carbon analysis

Labile organic carbon (LOC) fractions were determined using air-dried soil samples (< 2 mm) that had been pre-conditioned for at least 7 days. Particulate organic carbon (POC) was analyzed following the physical fractionation method of [17]. For this assay, 5 g of soil was suspended in 50 ml distilled water and shaken for more than 18 h, followed by sonication for 30 min using an ultrasonic processor (Branson, Brookfield, CT, USA). The suspension was then sieved through a 53 μm mesh, and the carbon content of the retained fraction was quantified using an elemental analyzer (Elementar, Variomax, Germany).

Permanganate-oxidizable carbon (POXC) was determined according to the procedure of [18]. Briefly, 2.5 g of soil was reacted with 20 ml of 0.02 M KMnO_4 stock solution, shaken for 2 min, and allowed to settle for 10 min. After sedimentation, 0.5 ml of the supernatant was diluted with 49.5 ml distilled water to prepare the analytical solution. Absorbance was measured at a wavelength of 550 nm using a UV/Vis spectrophotometer (SPECORD-200 PLUS, Analytik Jena, Germany), and POXC was calculated following the equations described by [18].

Microbial biomass carbon (MBC) was quantified using the chloroform fumigation-extraction method as described by [19]. In brief, 12 g of fresh soil was preincubated at 25 °C for 18 h before being placed in a desiccator. For fumigation, 50 ml chloroform was heated in a 100 ml beaker by water bath until boiling, and the vaporized chloroform was introduced into the desiccator under vacuum. After confirming stable vacuum conditions, soils were fumigated under dark conditions at room temperature for 24 h. Following fumigation, samples were extracted with 0.5 M K_2SO_4 by shaking for 1 h and then filtered. Filtrate was analyzed with a TOC analyzer (TOC-5050 A, Shimadzu Corporation, Japan). Non-fumigated controls were processed identically except for the fumigation step, and MBC was calculated as the difference between fumigated and non-fumigated extracts.

Maize growth and yield properties

To investigate plant growth characteristics, plants were randomly selected and measured height, total dry biomass, and grain yield. Total dry biomass and grain yield were determined by first recording fresh weight, then oven-drying samples at 70 °C to calculate dry matter content (%), which was subsequently multiplied by the corresponding fresh weight.

Statistical analysis

All experimental data were statistically analyzed using SPSS Statistics 26 (IBM SPSS, IBM Corp., Armonk, NY, USA). Treatment effects were evaluated through analysis of variance (ANOVA), and mean separations were performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level. The relationships between soil microbial activity and labile organic carbon were examined using Pearson's correlation coefficient.

Results and discussion

Soil properties

Soil pH, EC, and total nitrogen (T-N) were highest in the COM where cattle manure compost, characterized by high nutrient content and slow-release properties, was applied in Table 2. These results are consistent with previous findings that long-term application of livestock manure compost increases soil pH compared with chemical fertilizers [20]. The GM treatment also showed slightly higher soil pH and T-N than NPK treatment, and other chemical parameters exhibited similar trends, although differences were not statistically significant. Soil organic matter content in the organic material treatments was 24–87% higher than in the NF and 11–68% higher than in the NPK treatment. These results agree with previous studies that organic farming systems tend to accumulate more SOM than conventionally managed soils [21]. Also higher available P₂O₅ content of COM treatment (306.51 mg kg⁻¹) could be attributed to the elevated phosphorus input associated with livestock manure compost. Similarly, exchangeable cations were enriched under COM, whereas differences among the other treatments were negligible. Overall, these results indicate that cattle manure compost effectively improves

soil chemical properties, contributing to enhanced soil fertility and long-term soil health.

Soil microbial activity

Soil microorganisms play a crucial role in the decomposition of organic matter, and their activity is strongly influenced by environmental factors and organic inputs. Accordingly, microbial enzyme activities and soil respiration are widely recognized as sensitive indicators of soil biological functioning. In this study, five parameters; β-glucosidase (GLU), dehydrogenase (DHA), phosphatase (PHA), urease (URE), and soil respiration (RES), were examined (Fig. 1).

β-glucosidase (GLU) catalyzes the hydrolysis of cellulose and other β-glucosides into simple sugars, thereby driving soil carbon cycling [22]. Dehydrogenase (DHA) is involved in the biological oxidation of soil organic matter and serves as a general marker of microbial activity. In this study, GLU and DHA activities followed the order NF ≤ NPK < GM ≤ COM. Relative to NF, GLU activity was 55–91% higher in the organic treatments and 57–88% higher than in NPK. Similarly, DHA was elevated by 28–32% relative to NF and 37–41% relative to NPK. (Fig. 1) These findings are consistent with earlier studies reporting that organic matter addition microbial biomass and enzymatic activities [23, 24]. The higher GLU and DHA activities in the COM and GM treatments likely resulted from increased soil carbon inputs associated with long-term organic amendments [25, 26].

Phosphatase (acid phosphatase, PHA), which catalyzes the biochemical mineralization of organic phosphorus into inorganic forms, provides available P to plants and microorganisms [27]. PHA activity in this study ranked as COM (719.5 μg g⁻¹ h⁻¹) ≤ NK (932.5) < NPK (1283.9) ≤ GM (1372.2). Previous studies have shown that acid phosphatase activity decreased with increasing soil pH [28] lower PHA activity was observed in the COM treatment, where higher pH resulted from repeated manure applications.

Urease (URE), which hydrolyzes organic nitrogen into inorganic forms, plays a pivotal role in nitrogen cycling [29]. URE activity was observed in the order NF (165.9 μg g⁻¹ 2 h⁻¹) < NPK (230.2) ≤ GM (288.8) < COM (377.5). Relative to NF, URE activity was 74–128% higher in

Table 2 Soil chemical properties after maize harvest. Different letters indicate significant difference at *p* < 0.05. NF, no fertilizer; NPK, chemical fertilizer; COM, compost; GM, green manure

Treatment	pH	EC	T-N	T-C	Av.P ₂ O ₅	Exchangeable cations (cmol ⁺ kg ⁻¹)		
	(1:5)	(dS m ⁻¹)	(g kg ⁻¹)	(g kg ⁻¹)	(mg kg ⁻¹)	Ca	K	Mg
NF	6.3 ^b	0.13 ^c	0.93 ^c	8.51 ^c	15.93 ^b	5.01 ^b	0.39 ^c	1.58 ^b
NPK	5.5 ^c	0.20 ^b	1.04 ^c	9.45 ^c	33.27 ^b	4.25 ^c	0.41 ^c	1.37 ^c
COM	7.4 ^a	0.34 ^a	1.77 ^a	15.90 ^a	306.54 ^a	8.47 ^a	1.14 ^a	2.35 ^a
GM	6.2 ^b	0.16 ^{bc}	1.21 ^b	10.52 ^b	13.68 ^b	4.77 ^{bc}	0.74 ^b	1.43 ^{bc}

T-N, total nitrogen; T-C, total carbon; EC, electrical conductivity; Av. P₂O₅, available P₂O₅

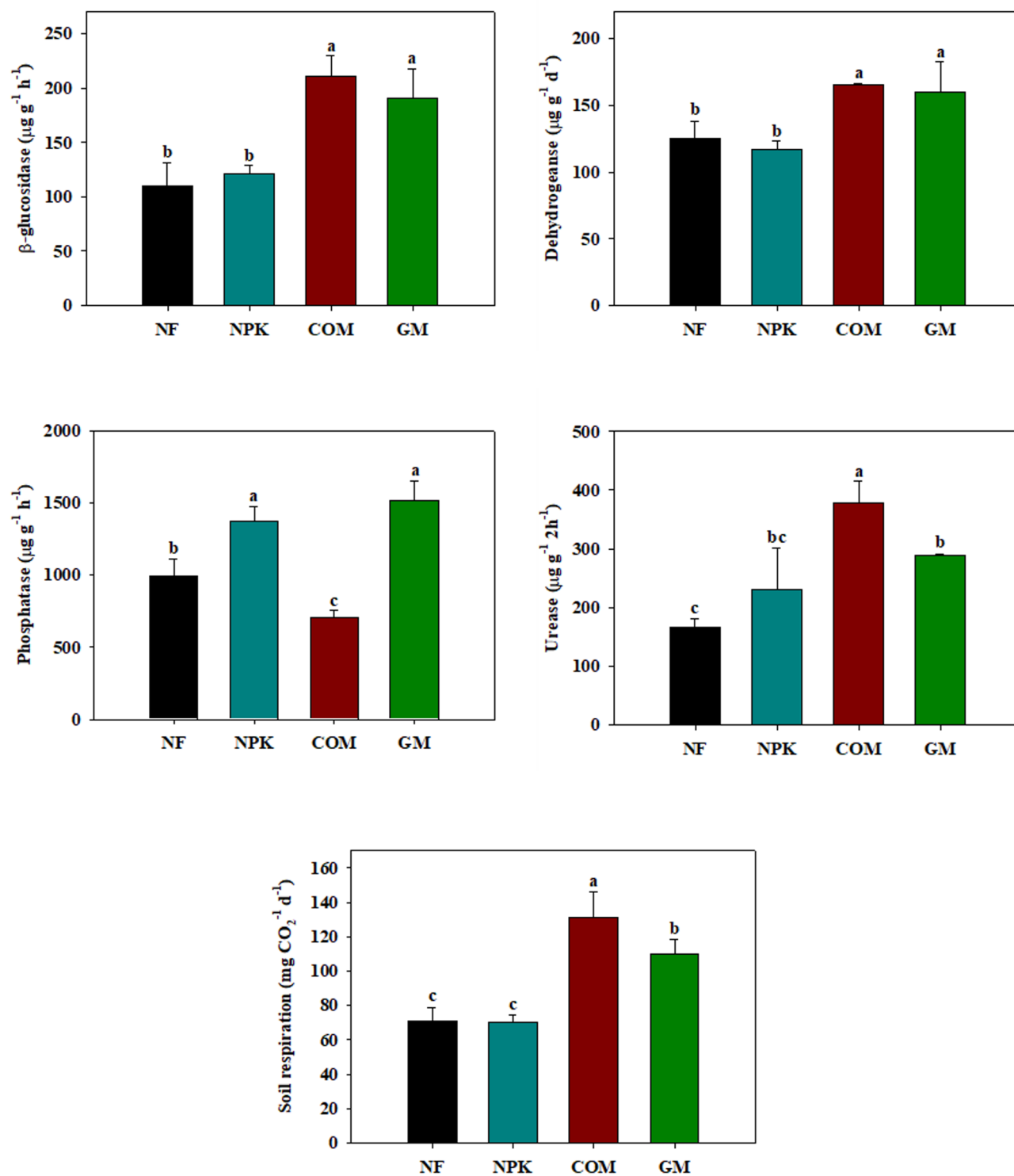


Fig. 1 Soil biological properties under different fertilizer treatments. Different letters indicate significant difference at $p < 0.05$. NF, no fertilizer; NPK, chemical fertilizer; COM, compost; GM, green manure

organic treatments and 25–64% higher than in NPK. The C: N ratio of organic inputs is a strong predictor of nitrogen mineralization potential, with lower ratios enhancing mineralization [30]. Accordingly, the absence of organic additions in NPK limited URE activity, whereas the low C: N ratio of COM (10.1) enhanced nitrogen availability, leading to the highest URE activity [31]. Although GM also had a relatively low C: N ratio and tended to promote URE activity, the difference from NPK was not statistically significant, in contrast to prior studies [29].

Soil respiration (RES), measured as CO₂ released during microbial oxidation of organic matter, is widely used as an indirect indicator of microbial activity. Similar to URE, the highest respiration rates were recorded in COM, reflecting rapid decomposition associated with its low C: N ratio, while GM also showed significantly higher respiration than NK and NPK.

Collectively, these results demonstrate that COM and GM treatment enhanced soil biological properties, fostering conditions conducive to microbial functioning and thereby improving overall soil health. Nonetheless, the differing responses of PHA and URE suggest that enzyme activities are governed by complex interactions among soil pH, nutrient dynamics, and microbial community structure. Further research is required to these interactions and their implications for soil biological health.

Soil labile organic carbon

Labile organic carbon (LOC) exists in soils in various forms and serves as an essential source of energy for microorganisms, playing a critical role in soil nutrient cycling. LOC is highly sensitive to soil physical, chemical, and biological properties and can be generated through multiple pathways [32]. In this study, Labile organic carbons were assessed in three fractions: physically derived particulate organic carbon (POC), chemically reactive permanganate-oxidizable carbon (POXC), and biologically mediated microbial biomass carbon (MBC). The effects of organic amendments on LOC fractions are presented in Table 3.

Both POC and POXC showed higher values in organic amendment treatments than in NF and NPK. Specifically,

Table 3 Labile organic carbon of soil under different fertilizer treatment. Different letters indicate significant difference at $p < 0.05$. NF, no fertilizer; NPK, chemical fertilizer; COM, compost; GM, green manure

Treatment	T-C g kg ⁻¹	POC g kg ⁻¹	POXC mg kg ⁻¹	MBC mg kg ⁻¹
NF	8.51 ^c	4.49 ^c	766.36 ^c	124.02 ^b
NPK	9.45 ^c	5.66 ^{bc}	1025.46 ^b	130.28 ^{ab}
COM	15.9 ^a	13.02 ^a	1213.64 ^a	216.55 ^a
GM	10.52 ^b	5.72 ^b	1041.82 ^b	194.69 ^{ab}

T-C, total carbon; POC, particle organic carbon; POXC, Permanganate oxidized carbon; MBC, microbial biomass carbon

POC was 190% higher than NF and 130% higher than NPK, and POXC was 58% higher than NF and 18% higher than NPK. Although GM exhibited higher values than NPK, the difference was not statistically significant, which may be attributed to the rapid decomposition of hairy vetch with a low C: N ratio, as well as the long-term return of weeds and maize residues. MBC also tended to be higher in organic treatments compared with NF and NPK. Previous studies have reported that long-term application of organic amendments increases MBC compared with chemical fertilization [33, 34], although no significant differences were observed in this study.

Correlation between soil microbial activity and labile organic carbon

To elucidate the relationships among soil chemical properties, microbial activity, and LOC, and to evaluate the potential of LOC as an indicator of soil health, Pearson correlation analysis was conducted (Fig. 2). Strong correlations between T-N, T-C and microbial activities, LOC fractions indicated that changes in soil T-N and T-C driven by organic amendments are major determinants of microbial activity and LOC content.

POC exhibited strong positive correlations with GLU ($r = 0.716, p < 0.01$), URE ($r = 0.805, p < 0.01$), and SRE ($r = 0.803, p < 0.01$). Similarly, POXC was significantly correlated with GLU ($r = 0.719, p < 0.01$) and URE ($r = 0.824, p < 0.01$). These findings suggest that both POC and POXC act as major carbon sources for microorganisms involved in organic matter decomposition and nitrogen cycling, thereby stimulating microbial activity [7, 35]. Accordingly, POC and POXC can be considered useful indicators for evaluating soil health.

MBC was positively correlated with GLU ($r = 0.834, p < 0.01$), DHA ($r = 0.753, p < 0.01$), and SRE ($r = 0.813, p < 0.01$), confirming its role as an integrative indicator of soil microbial activities and LOC factions [35]. The mean absolute correlation coefficients between microbial activities and LOC fractions followed the order POXC (0.59) < MBC (0.66) < POC (0.70), with POC showing the strongest associations. This highlights the role of POC as a primary energy source for microorganisms, exerting strong influence on microbial activity and underscoring its potential as an indicator of soil health. However, as exemplified by PHA, some indicators may not adequately capture enzymatic activity under specific soil conditions.

Maize growth and yield

The effects of organic materials on maize growth and yield are presented in Table 4. Grain yields in COM and GM were comparable to, or slightly higher than, those in NPK. Notably, total biomass production was greatest in COM, exceeding that of both NPK and GM. This outcome can be attributed to the higher phosphorus and

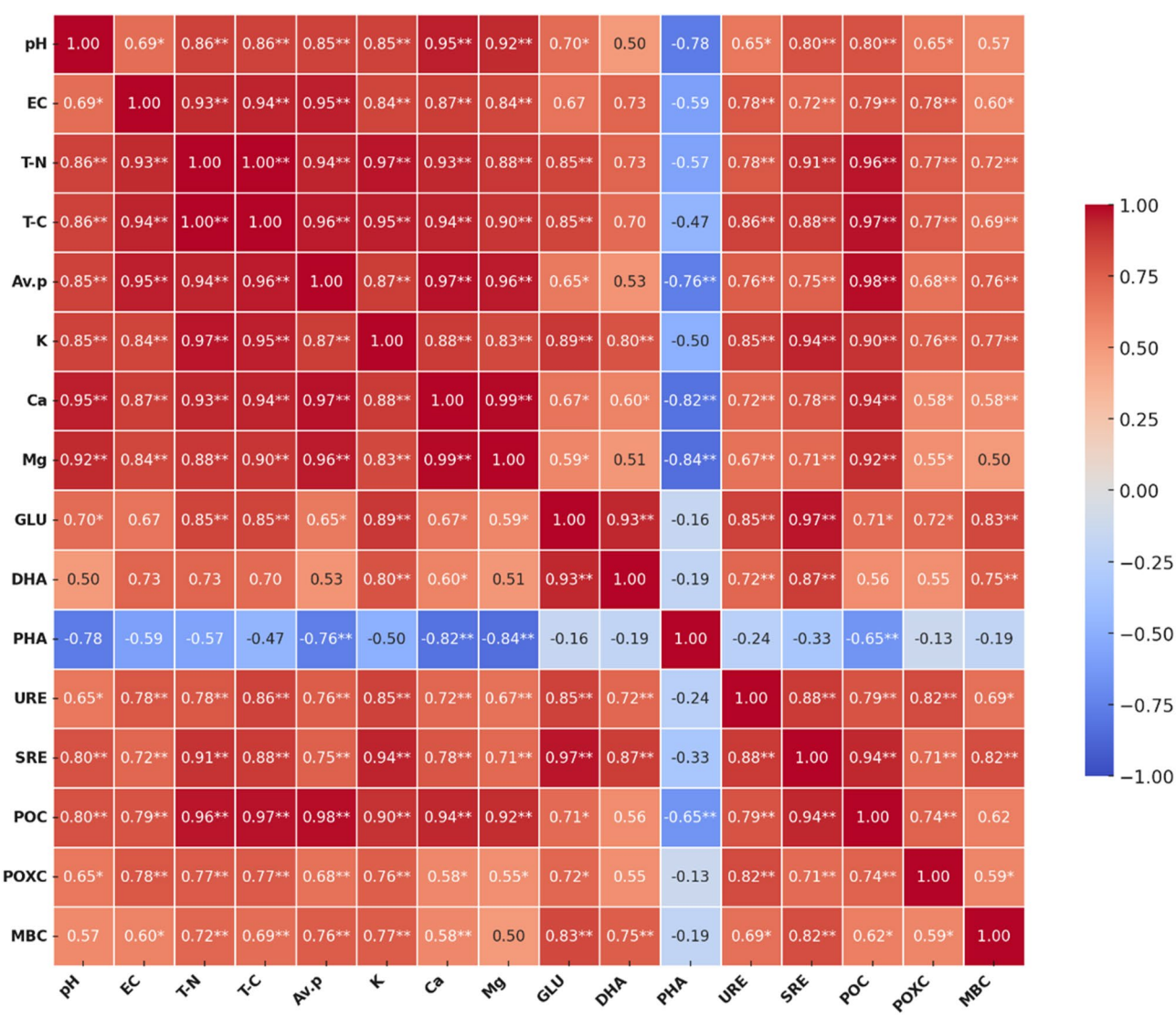


Fig. 2 Pearson correlation coefficients of the soil chemical, biological properties and labile organic carbon

Table 4 Maize growth and yield characteristics under different fertilizer treatments

Treatment	Plant height (cm)	Total biomass yield (kg 10a ⁻¹ , d.w.)	Grain yield (kg 10a ⁻¹ , d.w.)
NF	138.7 ^b	244.4 ^c	32.1 ^c
NPK	205.0 ^a	744.8 ^b	307.9 ^{ab}
COM	211.5 ^a	891.5 ^a	368.2 ^a
GM	212.6 ^a	757.6 ^b	293.6 ^b

Different letters indicate significant difference at $p < 0.05$. NF, no fertilizer; NPK, chemical fertilizer; COM, compost; GM, green manure; d.w, dry weight

calcium contents of COM, its slow-release characteristics that provided a sustained nitrogen supply, and the enhanced nutrient cycling driven by increased microbial activity (Fig. 1), all of which positively influenced crop growth [36]. These findings demonstrate that organic materials can maintain or even improve maize productivity relative to chemical fertilization, underscoring

their potential as sustainable nutrient sources for crop production.

This study examined the effects of different nutrient management practices on soil microbial activity and labile organic carbon (LOC) pools and evaluated their interrelationships to assess the potential of LOC as an indicator of soil health. The application of organic materials significantly enhanced microbial activities and LOC pools, leading to improvements in soil biological properties and overall soil health. There were strong positive correlations between microbial activities and LOC fractions, in particular Particulate Organic Matter (POC). Our findings suggest that POC and microbial enzyme activities are robust indicators of long-term organic management. However, rather than serving as stand-alone metrics, these indicators should be integrated into a comprehensive soil health assessment framework to

better monitor the synergy between carbon and nutrient cycling in sustainable agroecosystems.

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Author Contribution

YH Kim was the first author who mainly write this manuscript. YH Kim: Data curation, Writing-original draft, CR Lee: Data curation, Visualization, Writing-review & editing, NH An: Data curation, Visualization, Writing-review & editing, SM Lee: Data curation, Visualization, Writing-review & editing, HY Hwang: Supervision, Conceptualization, Writing-review & editing. HY Hwang manage and organize a full manuscript as a corresponding author. All authors participated in manuscript preparation, read and approved the final version.

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Data availability

Data will be provided on reasonable request.

Declaration

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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