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Dynamics of Nitrogen Mineralization by Organic and Inorganic Amendments Through Enzyme Activity of Microbial Community in Laboratory Incubation

Md. Tariful Alam Khan, Md. Billal Hossain Momen, Md. Rashedur Rahman Tanvir and Md. Robiul Islam*

Farming Systems Engineering Laboratory, Department of Agronomy and Agricultural Extension, University of Rajshahi, Rajshahi, Bangladesh

*Corresponding author: mrislam@ru.ac.bd

Abstract

Chemical fertilizers provide an immediate nitrogen supply but require repeated application at critical growth stages; however, excessive chemical fertilizer application harms the environment. In contrast, organic fertilizers release nitrogen gradually for a long time, and microbial fertilizers enhance nutrient availability. This study investigated the effects of integrating chemical nitrogen (CN), poultry manure (PM), and microbial fertilizer (MBF) on soil nitrogen availability and microbial activity. Eight treatments were applied: T_0 (control), T_1 (100% CN), T_2 (100% CN + MBF), T_3 (75% CN + 25% PM + MBF), T_4 (50% CN + 50% PM + MBF), T_5 (25% CN + 75% PM + MBF), T_6 (100% PM + MBF), and T_7 (100% PM). Soil nitrogen fractions, microbial biomass, enzyme activities, and phospholipid fatty acid (PLFA) composition were analyzed. Integrated treatments improved nitrogen availability compared to sole CN application, with T_4 showing the highest NO_3^--N accumulation. Additionally, T_4 increased total nitrogen, organic carbon, and microbial biomass, enhancing soil fertility. Enzymatic activities, including urease, catalase, invertase, and cellulase, responded positively to the integrated treatments, reflecting improved soil health. PLFA analysis revealed shifts in microbial community composition, highlighting the role of PM in promoting microbial diversity and biomass. These findings highlight that blending 50% CN and 50% PM with MBF balances immediate and sustained nitrogen release while stimulating microbial diversity and soil enzyme functions and improves overall soil health, making it a promising strategy for sustainable soil fertility management and reducing chemical fertilizer dependency.

Keywords: integrated fertilizer; microbial fertilizer; mineralization; PLFAs; poultry manure

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INTRODUCTION

The current advanced high-intensity crop production system has been characterized by the excessive use of chemicals such as herbicides, pesticides, and fertilizers to meet the increasing demand for agricultural and food commodities worldwide. This practice originated from the first Green Revolution (Hemathilake and Gunathilake, 2022). The positive relationship between crop yield and the use of chemical fertilizers has been demonstrated by previous studies (Guo et al., 2022; Peng et al., 2023). Nitrogen (N) is a crucial macronutrient that plays a vital role in various biological processes. It is essential for the synthesis of chlorophyll, nucleic acids, amino acids, proteins, and certain organic acids (Peng et al., 2021; Tariq et al.,

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2023). The indispensability of this particular entity is readily apparent, as it plays a crucial role in a wide array of biological and physiological processes. These include photosynthesis, biomass production, hormonal accumulation, carbohydrate allocation, plant root architectural growth, and the development of reproductive organs in plants (Liu et al., 2022; Wang et al., 2024).

In contrast, it should be noted that the excessive use of chemical nitrogenous fertilizer may not always yield the desired results in terms of enhancing crop productivity. This is primarily due to the heightened susceptibility to N losses through various mechanisms, including volatilization, denitrification, leaching, and eutrophication. These processes, which involve the emission of nitrogen oxides, contamination of underground water reserves, and pollution of surface water bodies, not only contribute to increased production costs but also pose significant environmental risks. These risks manifest in the form of soil degradation and compromised soil microbial diversity (Hoyt, 2022; Mansour et al., 2023).

To optimize crop productivity, mitigate environmental pollution, and achieve agricultural sustainability, it has become imperative to ensure the maintenance of soil fertility at a prescribed level or restore it when necessary (Sarkar et al., 2021). In agricultural practices, the mitigation of chemical fertilizers and pesticides can be accomplished by utilizing organic amendments (Syamsiyah et al., 2023). On the one hand, it is imperative to acknowledge that the proliferation of intensive animal husbandry practices has resulted in a significant increase in animal wastage, thereby emerging as a prominent contributor to global environmental pollution. On the contrary, the utilization of animal waste as organic manure has demonstrated its efficacy as a soil amendment, thereby enhancing crop productivity. This is attributed to the substantial presence of N, P, and other essential plant macro and micronutrients within the animal waste (Sudhakar, 2025). Additionally, this practice serves as an efficient means of waste disposal. Organic amendments, besides providing available forms of essential plant nutrients, enhanced soil water holding capacity, improved physical, chemical, and biological properties of soil, increasing soil microbial activity by increasing soil organic matter content (Singh et al., 2020; Kumar et al., 2021; Das and Ghosh, 2024).

The rapid expansion of the global poultry industry has resulted in the increased availability of poultry manure (Manogaran et al., 2022). The application of poultry manure and litter to soil has been shown to serve as a significant method for delivering vital plant nutrients, including N, P, K, Ca, and Mg, in significant quantities. This process facilitates the optimal growth of plants (Curtis et al., 2023). Additionally, the nutrient-rich composition of poultry manure and litter, coupled with its rapid-release characteristics and elevated organic C content, contributes to the restoration of soil fertility. The mineralization potential of poultry manure, specifically in terms of N and C, has been observed to have a significant impact on the quantity of net N mineralization that plants can uptake from manures (Tóth et al., 2023). Additionally, it has been noted that the presence of poultry manure can induce alterations in soil physiochemical processes, primarily by stimulating microbial activity through organic matter decomposition and enzymatic activities (Aboutayeb et al., 2024).

Microbial fertilizer, also known as biofertilizer, is of utmost importance in the intricate process of soil nutrient cycling by facilitating N fixation, P solubilization, and organic matter decomposition (Go Oco et al., 2024; Ma et al., 2025). The process of soil organic matter decomposition and subsequent release of easily accessible mineralized N in the soil is primarily facilitated by bacteria and fungi (Raza et al., 2023). The cycling of N through microbial processes is known to be significantly impacted by the content of soil organic matter (Lin et al., 2023). To gain a more comprehensive understanding of the intricate dynamics of nutrient availability in soil, it is imperative to thoroughly investigate the impact of microbial biomass functions on this process.

The study aimed to explore the enhanced mineralized N release capacity of poultry manure treated with microbial biofertilizer when applied to soil, addressing the knowledge gap in understanding how integrated applications of poultry manure, microbial biofertilizer, and chemical N influence N mineralization, microbial activity, and soil health. It sought to investigate the synchrony, N release ability, and fertilizer value of poultry manure across varying application rates, hypothesizing that combining these amendments would enhance N availability, improve soil microbial diversity, and promote enzymatic activities more effectively than individual applications. The primary objectives included assessing N mineralization and nitrification levels in poultry manure, both

individually and in combination with microbial biofertilizer. Additionally, the study aimed to evaluate the impact of integrated fertilizer application on the abundance and diversity of microbial communities pivotal in nutrient cycling processes, particularly through enzymatic activities, thereby ensuring N availability and continuous supply.

MATERIALS AND METHOD

Soil sampling/Collection

The soil used in this experiment was collected from the Agronomy Field Laboratory at the University of Rajshahi, Bangladesh. Geographically, the field is located at 88°38'36" E longitude and 24°22'36" N latitude at an elevation of 20 m above the sea level belonging to the High Ganges River Floodplain Agro-Ecological Zone (AEZ-11) of Bangladesh. Topsoil (0 to 15 cm) from five different points of the field was collected randomly using an auger and then mixed as a composite sample. The grasses and surface forest litter were removed from the sampling points before sample collection. The sample soil was collected in a zipper bag and carried to the laboratory, after that the natural field moist soil was sieved by a 4 mm strainer to eliminate coarse bricks, rocks, and other plant materials. The soil was first sieved and then thoroughly mixed to ensure uniformity before being promptly stored at 4 °C for subsequent use. Basic physical and chemical properties of the soil were assessed using a 500 g sub-sample, which was air-dried in the shade and subsequently passed through a 2-mm sieve. The soil type was identified as light Chernozem. Table 1 presents the fundamental physicochemical characteristics of the soil.

Poultry manure and microbial fertilizer collection

Poultry manure was gathered from the poultry farm in the Department of Veterinary and Animal Sciences at the University of Rajshahi, Bangladesh. The collected manure underwent a composting process, followed by crushing and sieving through a 1 mm mesh to ensure uniformity. Subsequently, the poultry manure was meticulously mixed to achieve optimal consistency. The physiochemical properties of poultry manure are shown in Table 2.

Microbial fertilizer

The microbial fertilizer utilized in this study was supplied by Beijing Liuhe Shenzhou Biotechnology Co. Ltd., with a guaranteed viable count of ≥ 2 million per gram, comprised of a blend of microorganisms, it was formulated using lignite as its primary component. High-throughput sequencing of the microbial biomass was conducted by Shanghai Meiji Biomedical Technology Company, employing the MiSeq sequencing platform. Following the manufacturer's guidelines, DNA extraction from 0.3 g of fresh soil was performed using the E.Z. N.A.® Soil DNA Kit provided by Omega Bio-teck Inc., Norcross, GA, USA. Three replicates of each soil sample's DNA were extracted and combined to generate a composite DNA sample. Subsequently, the concentration of the extracted DNA was determined using a NANO Quant (Tecan, Männedorf, Switzerland), followed by an examination of a 1% agarose gel.

The bacterial consortia underwent further analysis through sequencing of the V3-V4 hypervariable region of the 16S rRNA gene. Utilizing universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), the V3-V4 region was amplified. Meanwhile, the hypervariable regions of the fungal 18S rRNA gene were amplified using primers 817F (5'-TTAGCATGGAATAATRRAATAGGA-3') and 1196R (5'-TCTGGACCTGGTGAGTTTCC-3'), employing a thermocycler PCR system (GeneAmp 9700, ABI, USA). The PCR experiment was conducted in triplicate, with a reaction mixture comprising 5X FastPfu Buffer, dNTPs (2.5 mM), forward and reverse primers

Table 1. The basic physicochemical properties of the soil

Soil properties	Value
Bulk density (g cm^{-3})	1.34±0.04
Sand (%)	32.81±3.50
Silt (%)	39.00±4.47
Clay (%)	28.44±2.25
Soil pH (1:2.5 H_2O)	7.70±0.17
Organic C (g kg^{-1})	6.59±0.78
Total N (g kg^{-1})	0.54±0.06
NH_4^+-N (mg kg^{-1})	4.66±0.09
NO_3^--N (mg kg^{-1})	10.60±0.13
Available P (mg kg^{-1})	14.17±1.27
Available K (mg kg^{-1})	48.57±4.31
Cation exchange capacity (CEC) (cmol(+) kg^{-1} soil)	12.20±1.54

Note: The textural classification of the soil was according to the USDA classification system. Standard errors (±) of the means are added with the values, n = 4

(5 μM), FastPfu Polymerase, and template DNA. The PCR reactions followed a specific schedule: initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, elongation at 72 °C for 45 seconds, and a final extension step of 10 minutes at 72 °C. The results of the high-throughput sequencing of the beneficial functional flora within the microbial fertilizers utilized in this study are depicted in Figure 1a and 1b.

Laboratory incubation

The incubation experiment took place within the controlled environment of the Farming System Engineering Laboratory, housed within the Department of Agronomy and Agricultural Extension at the University of Rajshahi, Bangladesh, from November 2023 to January 2024. Fresh soil samples, each weighing 250 g and stored refrigerated for no longer than 15 days, were utilized for the experiment. These samples were transferred into 600 ml glass jars, the weights of which had been previously recorded. Deionized water was then added to each jar to adjust the soil moisture content to 60% of its water-holding capacity. Subsequently, the soil-filled jars underwent a pre-incubation phase lasting one week at 25 °C, aimed at stabilizing soil microbial activity. Throughout the incubation period, maintained at 25±2 °C, jars were arranged within the incubator according to a completely randomized design.

The experimental design involved two primary factors: treatments and time intervals. A total of 8 treatment combinations were established, delineated as follows: T_0 = Control (no chemical N and organic amendment), T_1 = 100% chemical N, T_2 = 100% CN + MBF, T_3 = 75% CN + 25% PM + MBF, T_4 = 50% CN + 50% PM + MBF, T_5 = 25% CN + 75% PM + MBF, T_6 = 100% PM + MBF, and T_7 = 100% PM, where CN was chemical nitrogen, MBF was microbial fertilizer, and PM was poultry manure. Throughout the incubation, which spanned 120 days following treatment application, observations were made at 12 predetermined time intervals: 1, 7, 14, 21, 28, 42, 56, 63, 84, 98, and 120 days by collecting soil using a small auger-like material carefully to avoid disturbing the remaining soil in the jar. The collected soils were small in amount and taken from different layers. Each treatment combination was replicated four times, with the entire experiment repeated twice.

Fertilizers were applied on an equivalent N basis at a rate of 200 mg N kg^{-1} soil. Phosphorus was sourced from $Ca(H_2PO_4)2{\cdot}H_2O$, while K was derived from K_2SO_4; both were applied to all experimental units, including the control, at rates of 90 mg P kg^{-1} and 60 mg K kg^{-1}, respectively. Additionally, microbial fertilizer was incorporated into the soil at a rate of 10 g kg^{-1}. The treatments were applied to the soil following the experimental design and thoroughly mixed. Each jar was then covered with a perforated transparent lid to facilitate natural gas exchange, with the weight of each jar recorded after covering. Deionized water was added at 2-day intervals as necessary to maintain the field capacity, replenishing any weight loss exceeding 0.05 g. Importantly, soil disturbance such as shaking or stirring was avoided to maintain undisturbed conditions throughout the incubation period.

Extraction of soil sample and analysis

Samples were collected from each treatment replication unit and incubated over various time intervals to assess total mineral nitrogen (TMN), ammonium N (NH_4^+-N), and nitrate N (NO_3^--N) levels. Each fresh soil sample was divided into two portions. One portion was stored in a plastic bag at -4 °C to analyze mineral N, microbial biomass nitrogen (MBN), microbial biomass carbon (MBC), enzyme activities, and phospholipid fatty acids. The other portion was air-dried, stored in plastic bags, and kept at 4 °C in a refrigerator for the measurement of total N,

Table 2. Chemical properties of poultry manure used in the study

Chemical properties	Concentration
Total N (g kg^{-1})	18.68±3.1
Total P (g kg^{-1})	8.17±1.4
Total K (g kg^{-1})	5.41±1.6
Total C (g kg^{-1})	324.20±23.5
C:N	17.34±2.6
Organic matter (g kg^{-1})	602.50±24.8
Ca (g kg^{-1})	32.00±4.0
Mg (g kg^{-1})	5.20±0.82
Fe (mg kg^{-1})	1,087.00±106.8
Zn (mg kg^{-1})	376.20±24.4
Mn (mg kg^{-1})	564.50±19.6
Cu (mg kg^{-1})	421.00±20.7
Cellulose (g kg^{-1})	81.90±2.8
Lignin (g kg^{-1})	63.40±3.3
Polyphenol (mg kg^{-1})	256.70±2.5

Note: Values presented after ± are standard error of the means (SEM), n = 4

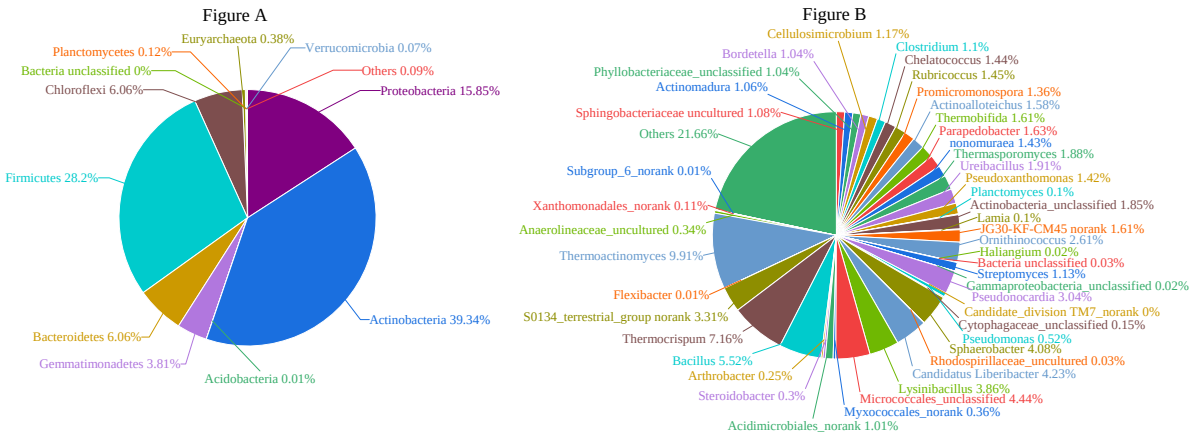

Figure 1. Available microbes in the microbial fertilizer, (a) genus level and (b) phylum level

organic C, and pH. Soil pH was determined by creating a 1:2.5 soil:water suspension. NH_4^+-N and NO_3^--N levels were measured using a continuous flow analyzer (AA3 type, company: Seal, Norderstedt, Germany). Soil organic carbon (SOC) was calculated using the Walkley-Black technique (Nelson and Sommers, 1982).

Chloroform fumigation-extraction was employed to determine the C and N content of soil microbial biomass (Vance et al., 1987). For this, 20 g of thoroughly mixed soil was directly extracted with 80 ml of 0.5 M K_2SO_4 by shaking for 30 minutes at 180 revolutions per minute. Another 20 g of soil was similarly extracted after chloroform fumigation for 24 hours. The content of total C in the extracts was determined using a 2100 TOC/TIC analyzer (Analytik Jena, Germany). Biomass was determined based on the difference in K_2SO_4 extract between fumigated and non-fumigated soil samples, using conversion factors of 0.54 for MBN (kEN) and 0.45 for MBC (kEC) in Equation 1 and Equation 2, respectively (Smolander and Kitunen, 2002).

$$MBN = \frac{E_N}{k_{EN}} \quad (1)$$

$$MBC = \frac{E_C}{k_{EC}} \quad (2)$$

The designations of "E_C" and "E_N" denote the discrepancies in organic C and total N levels between fumigated and non-fumigated treatments, respectively. The soil underwent a 7-day pre-incubation at 20 °C in darkness, with its moisture content set to 55% of its water-holding capacity to determine soil respiration (SR). Carbon dioxide (CO_2) emissions from the moist soil were consistently measured throughout this period. Subsequently, over the subsequent 7-day period, CO_2 was quantified utilizing a NaOH trap, after which hydrochloric acid (HCl) was employed to titrate the concentration (Jenkinson et al., 2004).

Soil enzyme activity

The urease activity was analyzed by the Phenol-sodium hypochlorite colorimetric method (Paul, 2014), which was determined by incubating 1 g soil for 24 hours and indicating the amount of NH_3-N mass (mg) in it. The catalase activity was determined using the titration method, measured by the depletion (ml) of 0.1 mol l^{-1} $KMnO_4$ during a 20-minute incubation of 1 g of soil. Invertase enzyme activity was assessed using a universal modified buffer, followed by a colorimetric technique involving 3,5-dinitro salicylic acid monohydrate (Wu et al., 2008). After a 24-hour soil incubation, the results were expressed as the mass (mg) of glucose per gram of soil. The 3,5-dinitro salicylate colorimetric method was used to detect cellulose enzyme activity, where the soil sample was incubated for 24 hours at 30 °C with acetate buffer (50m~, pH 5.5), carboxymethyl cellulose (CMC), and toluene to determine the produced rate of reducing sugars (mg glucose) generated by decomposition of cellulose in 1 g soil (Deng and Tabatabai, 1994).

Phospholipid fatty acid (PLFA) analysis

Every time just after sampling, the soil was preserved and freeze-dried at -20 °C till analysis. A total of 4 g soil was used to extract PLFAs. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the extracted sample, and GC-MS was thermos focus GC and thermos dual stage quadrupole (DSQ) MS in electron ionization mode. The labeling of fatty acids was done as X: Y ω Z, where 'X' represents

the C atom number of the chain, 'Y' shows the number of double bonds, and the C atom number starting from the end molecule of methyl to the 1st unsaturated bond was represented by 'Z' (Peacock et al., 2001; Fraterrigo et al., 2006). The adjuncts are defined as follows: *a* denotes anteiso, *i* represents iso, *cy* indicates cyclopropyl branching, and *d* refers to dicarboxylic fatty acids, while *br* signifies an unknown branching pattern, and *ME* denotes the position of the methyl group.

The OH groups in the OH fatty acid located in the 2 and 3 positions were labeled by α and β, respectively, and the geometry of cis and trans was indicated by c and t. Various fatty acids imply different microbial groups: Gram-negative bacteria have cyclopropyl fatty acids, whereas Gram-positive bacteria have branched fatty acids (e.g., iso and anteiso) (Zelles, 1999; Balser and Firestone, 2005); fungi and bacteria were typically indicated by monounsaturated fatty acids (Zelles, 1999; Fraterrigo et al., 2006); and except for cyanobacteria, polyunsaturated fatty acids were characteristic of eukaryotes. Actinomycetes were represented by fatty acids with methyl branching on the tenth C atom (Zelles, 1999). The concentration of PLFA for different microbial groups, including the total PLFA, was calculated. For the Gram-positive bacteria, by the summation of iC15:0, aC15:0, iC16:0, aC16:0, iC17:0, and aC17:0 was calculated. The summation of cyC17:0 and cyC19:0 fatty acids represented the Gram-negative bacteria. The summation of Gram-positive bacteria, Gram-negative bacteria, C15:0, and C17:0 illustrated the total bacterial community. The sum of C18:2 ω 9, 12c, C18:1 ω 9c, and C18:3 ω 9, 12, and 15c represented fungi, and C16:1 ω 11 represented arbuscular mycorrhizal fungi (AMF). By summing 10Me16:0 and 10Me18:0, actinomycetes were determined.

Statistical analysis

Through the use of two-way analysis of variance (ANOVA) using the SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA), the effects of various treatments on the different forms of N and C present in the soil, MBN, MBC, respiration, enzyme activity, and PLFA concentration were examined. The Pearson linear technique was used to model the connections between the SOC fractions and soil respiration as well as soil enzyme activity. The least significant difference at a 0.05 or 0.01 level of probability was used to compare the means of various treatments. Sigma plot 13.0 for Windows (Systat Software, San Jose, CA) was used for the graphic rendering. The entire data set was shown as means with standard errors (SE) of four replications.

RESULTS AND DISCUSSION

Alteration in the soil nitrification and mineralization

The net cumulative NO_3^--N concentration under different treatments significantly varied with the incubation time and N fertilization treatments (Figure 2a). Among the various treatments, T_4 showed the highest NO_3^--N accumulation, from day 42 (78 mg kg^{-1}) to the end of the experiment (124 mg kg^{-1}), which was significantly higher than the rest of the treatment (during most of the period of incubation). The NO_3^--N accumulation in the combined treatments T_3, T_4, and T_5 was 53%, 62%, and 43%, respectively, of the total applied N. On the other hand, till day 35, the NO_3^--N accumulation in the treatments T_1 and T_2 was the highest, but after day 35, the increasing rate of NO_3^--N in these treatments became slow and at the end (day 120) the NO_3^--N was 89 and 99 mg kg^{-1} soil, respectively (Figure 2a).

On the other hand, soil amended with only organic N source in treatments T_6 and T_7 from the beginning to the end the NO_3^--N accumulation rate slowly increased and showed the lowest concentration compared to other treatments except control over the incubation period which finally was 80 and 65.5 mg kg^{-1}, respectively (Figure 2a). Treatment T_4 showed the highest nitrification rate of 1.04 mg kg^{-1} soil day^{-1}, which was 78% higher than the T_1, and $T_3 > T_2$ was the following lower order. Among the amended soil, the lowest nitrification was found in the treatment T_7, which was 49% lower than the T_4.

A similar trend was also found in the case of N mineralization, and soil amended with different types and proportions of organic and inorganic N sources displayed distinguishable rates of mineralized N over different days of incubation. Although during the initial stage, the highest concentration of mineralized N 157 and 172 mg kg^{-1} was observed on day 14 in the soil amended with 100% CN under the treatments T_1 and T_2, respectively, thenceforward, the mineralized N concentration (Figure 2b) in this treatment was declining day by day to the end and finally decreased almost 50% about the peak point

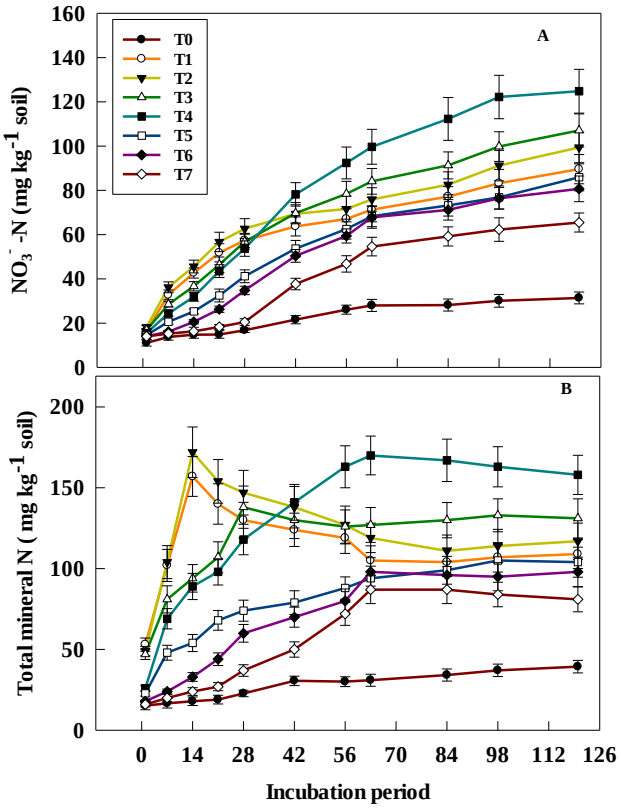

Figure 2. Changes in net cumulative nitrification and mineralization of N in response to the addition of sole application of chemical N, poultry manure, and microbial fertilizer and their integrated application with various combinations (equivalent to 200 mg N kg^{-1}) which was incubated at 25 °C for 120 days. (a) Total nitrification during the incubation period, (b) Total mineralization during incubation

Note: Bars indicate standard errors (n = 4). T_0 = Control, T_1 = 100% CN, T_2 = 100% CN + MBF, T_3 = 75% CN + 25% PM + MBF, T_4 = 50% CN + 50% PM + MBF, T_5 = 25% CN + 75% PM + MBF, T_6 = 100% PM + MBF and T_7 = 100% PM

(Figure 2b). Contrarily, at the earlier phase, the N mineralization process was comparably negligible in the treatments with no CN and only amended with 100% PM and 100% PM + MBF (Figure 2b). The mineralization slowly increased on day 63 and thereafter remained almost stable to the end day, also showing comparably the lowest mineralized N concentration over the incubation period.

In the sole 100% PM treatment, the highest concentration of 87 mg kg^{-1} mineralized N was observed on day 63, which remained statistically unchanged on day 84 and later declined a little bit with time. This result indicated that applied N in the form of PM released 43.5% N to the total mineralized N pool in treatment T_7 (Figure 2b). Interestingly, a higher concentration of net cumulative mineralized N was observed in the combination treatments of T_3 and T_4 on the end day, which was significantly higher than in the individual application of the CN and PM. Just after day 42 to the end day, treatment T_4 revealed the highest cumulative net mineralized N reached its peak point on day 63 with a mineralized N concentration of 160 mg kg^{-1}, which finally was 148 mg kg^{-1} at the end, and that was significantly higher than the all-other treatments (Figure 2b). Compared to the control, treatment T_4 showed 58% and 41% more mineralized N on days 63 and 120, respectively (Figure 2b).

The concentrations of total N in the soil, as well as its various fractions, were observed to increase when the addition of MBF accompanied the integrated application of PM and CN. Additionally, the impact of PM on these N parameters was found to be significantly greater as the proportion of PM in the mixture increased, reaching its peak at a ratio of 50% PM and 50% CN when supplemented with MBF. These phenomena may be associated with the slow

release of organic N inputs in PM, which enhances microbial activity (Liu and Zhang, 2023). Poultry manure provides N in organic form, while chemical fertilizers supply N primarily as ammonium nitrate (NH_4NO_3) or urea. The application of both sources enhances soil N levels by directly adding their respective N contributions (Iqbal et al., 2022).

The T_1 and T_2 showed a significant increase in NO_3^--N accumulation. This increase was observed mainly during the initial stage, with approximately 60 to 66% of total nitrification occurring within the first 28 days. However, the rate of NO_3^--N increased in these two treatments slowed down afterward. By the end of the incubation period, approximately 40% and 44% of the applied urea N had been nitrified in treatments T_1 and T_2, respectively (Figure 2a). Similarly, during the early phase, the concentration of mineralized N in the soil treated with 100% CN (T_1 and T_2) was the highest (Figure 2b). This increase was due to the excessive addition of NH_4^+-N given by the NH_2-CO-NH_2 fertilizer, which is CN, as shown in Figure 2b. As a result, the concentration of mineralized N in these two treatments decreased over time, possibly due to the ongoing process of nitrification-denitrification (Daly et al., 2023), which includes changes in the immobilization process (He et al., 2023).

The levels of MBN content in treatments T_1 and T_2 were consistently the lowest during the incubation period. This can be attributed to the fact that only CN was administered in these treatments, which resulted in stronger acidification effects due to the CN fertilizer (Souza et al., 2023). On the other hand, the soil's total N content in both treatments was also found to be the lowest after the incubation period. This could be attributed to the fact that a significant amount of N was converted into minerals during the initial phases due to the CN fertilizer (Wang et al., 2023). In contrast, the concentration of NO_3^--N in treatments T_6 and T_7 consistently decreased during the incubation time. In the end, it was found that 35% and 32% of the applied PM was nitrified in treatments T_6 and T_7, respectively (Figure 2a).

The soil, which had been treated with T_7 and T_6, underwent gradual mineralization during the initial phase. However, starting from day 42, the mineralization process accelerated and reached its peak at day 63. After that, there were no significant changes in the mineralization rate over the latter days. In treatments T_6 and T_7, the mineral N content peaked at 87 and 94 mg kg^{-1}, respectively. This indicates that 43% and 47% of N was released from PM through mineralization by day 63 (Figure 2b). Earlier studies reported that the highest N mineralization rate from poultry manure was in different ranges, e.g., 25 to 61% (Grijalva et al., 2010), 51% and 53% (Qafoku et al., 2001), 44% (Moore et al., 2010), 61% (Alizadeh et al., 2012) and 42% (Shah et al., 2013). The differential mineralization rate of PM could be due to feed type, chemical composition of PM, type and proportion of litter dropping, handling of the manure, soil pH, microbial activity, temperature, soil aeration, and moisture content. The rate at which poultry manure mineralized was controlled by soil characteristics and moisture content. Lower mineralization rates resulted from the immobilization of C from poultry manure in soil with a greater organic C content (SOC) and clay loam soil texture. Conversely, in soils with sandy and loamy sand textures, just 2% of the C in the poultry manure was mineralized (Kaur et al., 2023).

A negative correlation was found between the total N mineralization and the total N in the soil. This correlation showed that the total N in the soil was related to the applied materials' capacity for mineralization, which is another way of saying that higher soil total N values are typically associated with lower mineralization rates. It is interesting to note that applying CN, PM, and MBF together can improve the release of N into the soil's mineralized N pool compared to applying CN and PM alone. In addition to improving soil health and supporting sustainable N management, the combining strategy gives plants both readily available and sustained sources of N (Prado et al., 2023). This could be a result of all the CN being hydrolyzed at the beginning and most of the PM being mineralized as enzyme activity increased.

In contrast to the solitary application of CN or PM, the study found that the use of integrated fertilizers, such as 75% CN + 25% PM + MBF, 50% CN + 50% PM + MBF, and 25% CN + 75% PM + MBF, considerably boosted the accumulation of NO_3^--N in the soil mineralized N pool. This could be due to massive nitrification from organic and inorganic N sources. Moreover, adding MBF to the soil increases its MBN and MBC levels, which in turn stimulates microbial activity and accelerates the process of nitrification and mineralization, which are two ways that N is transformed in the soil (Li et al., 2023). A prior study found that the buildup of NO_3^--N under PM

when mixed with residues from white clover plants was 38% and under PM when combined with urea N, 69% (Abbasi and Khizar, 2012). This outcome demonstrated how, under field conditions, a single application of synthetic mineral N in various forms releases an available form of N quickly in the beginning stage, which is typically vulnerable to losses through runoff, eutrophication, volatilization, denitrification, and leaching. Because bacteria immobilize soil's mineral N during the breakdown of organic matter, this sort of N loss can be reduced by utilizing organic N in conjunction with mineral N and MBF supplementation. Thus, net N mineralization slows down in organic matter, resulting in long-term stability of NH_4^+-N in the soil and postponed nitrification, which eventually reduce N loss through denitrification and leaching. Furthermore, compared to the application of CN alone, a beneficial rise in MBN and total N was also noted in the combined treatment (Vanlauwe et al., 2015).

Alteration in soil total N and organic C

Different N sources significantly altered the total N concentration and organic C content in the soil. The change patterns of soil total N and SOC were similar during the whole incubation period, decreasing trend over time. The highest concentration of soil total N was observed in the treatment where 100% PM was added in T_6 and T_7, which was 26% and 19% higher, respectively, compared to T_1. The following sequential order of lower concentrations was $T_5 > T_4 > T_3$ (Table 3). The lowest total N concentration was exhibited in the soil amended with sole 100% CN. Similarly, at the beginning of the incubation, significant ($p \leq 0.05$) variations in SOC were exhibited among the different treatments, and the soil amended with poultry manure and microbial fertilizer sole or integrated with CN displayed higher SOC compared to the treatment which is amended with 100% CN (Table 4).

Treatment T_6 showed the highest SOC over the incubation period, which was 14.06 and 11.16 g kg^{-1} at the beginning and end, respectively (Table 4). A statistically non-significant variation was observed between treatments T_6 and T_7. The lowest SOC was found in treatment T_1, which was 8.52 g kg^{-1} at the initial phase of the incubation and almost remained constant (having no significant changes) but slightly decreased at the end (Table 4). Interestingly, SOC in the different amended soils continuously decreased over time, and at one day (day 120), a 19 to 25% reduction in the SOC was observed among the treatments.

Applying PM can act as a direct supply of C for the soil's organic matter (Cardarelli et al., 2023). According to this study, the amount of total organic C in the soil rose as the applied PM rate increased (Table 3). The organic material present in poultry manure acts as a readily accessible nourishment for soil microorganisms. As the microorganisms break down the manure, they secrete enzymes that further degrade organic substances and release extra C into the soil. The heightened microbial activity also results in the creation of humic compounds, which are enduring forms of organic C that can endure in the soil for generations (Amorim et al., 2022; Mindari et al., 2025).

Again, the quantity of total organic carbon (TOC) in the soil exhibited alterations as time progressed. The study observed a decrease in TOC, which may be attributed to the mineralization of soil organic matter. This occurs when bacteria utilize C as energy for the decomposition of organic matter. The soil's organic C saw a considerable loss within the first 63 days. However, after that period, it either stayed stable or decreased without reaching statistical significance (Table 3). The increased accumulation of organic C in treatments T_6 and T_7 may be attributed to a decrease in the mineralization of organic manure. Regularly incorporating organic matter into the soil can increase its organic C content (Canisares et al., 2023). The initial stage of the incubation period exhibited the lowest concentration of organic C in soil treated with either CN or CN + MBF, and this level remained statistically constant throughout. The decline in organic C content can be attributed to the inadequate quantity of basic organic C and the absence of particulate matter throughout these treatments.

Alteration in the soil microbial biomass and respiration

Soil MBN and MBC almost displayed similar trends, and significant differences were reflected among different treatments. Throughout the incubation period, MBN varied from 10 to 123.3 mg kg^{-1}, consistently reaching its highest level in treatment T_6, followed by T_4 and T_7 in decreasing order (Figure 3a). MBN reached its peak point (123 mg kg^{-1}) on day 56 in treatment T_6, which was 4 times higher than the control on a similar day. A significantly decreased level of MBN was observed in treatments T_1 and T_2 from the initial

Table 3. Changes in the total N of the soil (g kg^{-1}) were amended with the sole application of chemical N, poultry manure, and microbial fertilizer and their integrated application with various combinations (equivalent to 200 mg N kg^{-1}), which was incubated at 25 °C for 120 days

Treatment	Incubation time (Day)										
	1	7	14	21	28	42	56	63	84	98	120
T_0	0.53^{b}	0.53^{b}	0.52^{d}	0.52^{e}	0.51^{e}	0.49^{d}	0.48^{c}	0.47^{e}	0.46^{e}	0.46^{e}	0.45^{e}
T_1	0.73^{a}	0.73^{a}	0.70^{ab}	0.66^{cd}	0.65^{cd}	0.63^{c}	0.61^{b}	0.60^{d}	0.52^{de}	0.51^{de}	0.51^{de}
T_2	0.74^{a}	0.71^{a}	0.64^{c}	0.61^{d}	0.60^{d}	0.61^{c}	0.59^{b}	0.59^{d}	0.57^{cd}	0.56^{cd}	0.56^{cd}
T_3	0.76^{a}	0.73^{a}	0.66^{bc}	0.65^{cd}	0.63^{cd}	0.62^{c}	0.61^{b}	0.61^{cd}	0.60^{c}	0.60^{bc}	0.59^{bc}
T_4	0.75^{a}	0.75^{a}	0.74^{a}	0.73^{ab}	0.72^{ab}	0.71^{ab}	0.70^{a}	0.66^{bc}	0.62^{c}	0.61^{bc}	0.60^{bc}
T_5	0.76^{a}	0.76^{a}	0.72^{a}	0.69^{bc}	0.66^{bc}	0.65^{bc}	0.64^{b}	0.64^{bcd}	0.63^{bc}	0.63^{b}	0.62^{b}
T_6	0.75^{a}	0.75^{a}	0.75^{a}	0.74^{a}	0.74^{a}	0.73^{a}	0.74^{a}	0.73^{a}	0.72^{a}	0.71^{a}	0.69^{a}
T_7	0.74^{a}	0.74^{a}	0.74^{a}	0.73^{a}	0.72^{a}	0.72^{a}	0.70^{a}	0.69^{ab}	0.68^{ab}	0.64^{b}	0.63^{b}

Note: Lowercase lettering is used to show the significant differences between different types of treatments at $p < 0.05$ level. T_0 = Control, T_1 = 100% CN, T_2 = 100% CN + MBF, T_3 = 75% CN + 25% PM + MBF, T_4 = 50% CN + 50% PM + MBF, T_5 = 25% CN+ 75% PM + MBF, T_6 = 100% PM + MBF, T_7 = 100% PM

Table 4. Changes in the organic C content of the soil (g kg^{-1}) were amended with the sole application of chemical N, poultry manure, and microbial fertilizer and their integrated application with various combinations (equivalent to 200 mg N kg^{-1}), which was incubated at 25 °C for 120 days

Treatment	Incubation time (Day)										
	1	7	14	21	28	42	56	63	84	98	120
T_0	6.59^{f}	6.58^{f}	6.56^{e}	6.54^{f}	6.51^{f}	6.49^{e}	6.47^{f}	6.45^{f}	6.43^{g}	6.42^{g}	6.41^{g}
T_1	6.61^{f}	6.61^{f}	6.60^{e}	6.59^{f}	6.57^{f}	6.53^{e}	6.53^{e}	6.52^{e}	6.50^{f}	6.48^{fg}	6.45^{fg}
T_2	6.64^{f}	6.63^{f}	6.62^{e}	6.60^{f}	6.57^{f}	6.56^{e}	6.54^{e}	6.53^{e}	6.51^{f}	6.50^{f}	6.48^{f}
T_3	9.73^{e}	9.68^{e}	9.61^{d}	9.58^{e}	9.54^{e}	9.38^{d}	9.21^{d}	8.89^{d}	8.08^{e}	7.43^{e}	7.27^{e}
T_4	10.18^{d}	10.08^{d}	9.99^{c}	9.86^{d}	9.68^{d}	9.43^{d}	9.23^{d}	8.90^{d}	8.54^{d}	8.30^{d}	8.17^{d}
T_5	11.59^{c}	11.52^{c}	11.50^{b}	11.48^{c}	10.99^{c}	10.56^{c}	10.18^{c}	9.92^{c}	9.65^{c}	9.39^{c}	9.22^{c}
T_6	14.06^{a}	13.88^{a}	13.76^{a}	13.69^{a}	13.53^{a}	13.19^{a}	12.34^{a}	12.01^{a}	11.88^{a}	11.39^{a}	11.16^{a}
T_7	13.84^{b}	13.80^{b}	13.73^{a}	13.35^{b}	13.14^{b}	12.88^{b}	12.17^{b}	11.26^{b}	11.15^{b}	11.05^{b}	10.79^{b}

Note: Lowercase lettering is used to show the significant differences between different types of treatments at $p < 0.05$ level. T_0 = Control, T_1 = 100% CN, T_2 = 100% CN + MBF, T_3 = 75% CN + 25% PM + MBF, T_4 = 50% CN + 50% PM + MBF, T_5 = 25% CN+ 75% PM + MBF, T_6 = 100% PM + MBF, T_7 = 100% PM

phase to the end of the incubation. A clear variation was observed for MBC among the treatments during the study period. Soil MBC ranged from 51 to 183 mg kg^{-1}, with the highest values consistently observed in treatment T_6, peaking on day 63. Treatment T_7 followed in decreasing order throughout the incubation period (Figure 3b). Until day 56, MBC in treatment T_4 was higher than in T_5; however, MBC in T_4 declined afterward. Similar to the MBN, treatments T_1 and T_2 always exhibited a lower MBC content, which showed little increasing trend on day 28, before declining toward the end of the incubation period.

Soil with different fertilization treatments showed diverse respiration rates and higher cumulative CO_2 production compared to those in the control treatment (Figure 3c). A significantly higher respiration rate was observed in treatment T_6, followed by $T_7 > T_5 > T_4$ in decreasing order compared to the other treatments. The respiration rate in T_6 was 4.1 and 3.7 times higher than in control at days 63 and 120, respectively. Similarly, compared to treatment T_1 (only CN),

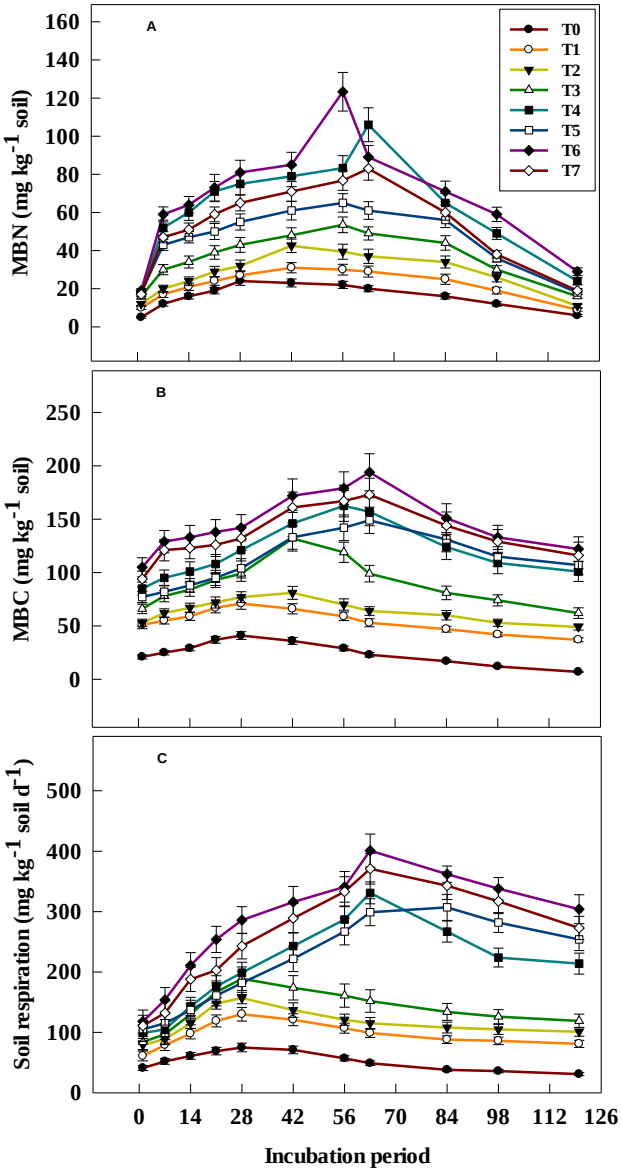

Figure 3. Soil microbial biomass and respiration rate. (a) Soil MBN, (b) Soil MBC, and (c) Soil respiration rate in different treatments over the incubation period at different days' interval

Note: Bars indicate standard errors (n = 4). T_0 = Control, T_1 = 100% CN, T_2 = 100% CN + MBF, T_3 = 75% CN + 25% PM + MBF, T_4 = 50% CN + 50% PM + MBF, T_5 = 25% CN+ 75% PM + MBF, T_6 = 100% PM + MBF, T_7 = 100% PM

3.8 and 3.4 times more respiration rate was observed in treatment T_7 (only PM) at day 63 and end day, respectively (Figure 3c). Like MBC, until day 63, the respiration rate in treatment T_4 was higher than that in T_5, but in the later phase, it declined compared to the respiration rate in T_5.

Enzyme activities in soil

Different sources of N significantly affected the enzyme activities in the soil over time. All four enzymatic activities, including urease, catalase, invertase, and cellulase, showed similar changing patterns over the experiment. During the initial stage at day 7, there were no significant differences observed among the treatments except for invertase enzyme activities; later phase differences were found among the treatments significantly. Treatment T_6 always showed the highest in enzymatic activities over the incubation time for all four enzymes, and $T_4 > T_7 > T_5 > T_3 > T_2 > T_1$ was in the following order (Figure 4 a-d). From the beginning of the incubation to day 63, an increasing pattern was observed, and the highest enzymatic activity was detected at day 63. After day 63, a decreasing orientation was found. Treatments T_1 and T_2 always showed the lowest enzymatic activities in the experiment. Soil MBN, MBC, SOC, and respiration rates were strongly correlated with the enzymatic activities at day 63 and at the end of the incubation (Table 5).

Alteration in the PLFA concentration

The changes in PLFA concentrations on day 63 and day 120 were analyzed. The total PLFA concentration peaked on day 63 and gradually decreased over the incubation period. The control treatment illustrated the lowest total PLFA concentration over the incubation period. In all the treatments, T_6 represents the highest total PLFA concentration (19.87 nmol g^{-1} soil), which was 135.99% higher than the control at day 63 (Figure 5 a-e). The incubation period and experimental treatments both have significant effects on the total PLFA concentration.

According to the fatty acid biomarker, the concentration of bacterial biomass was dominant

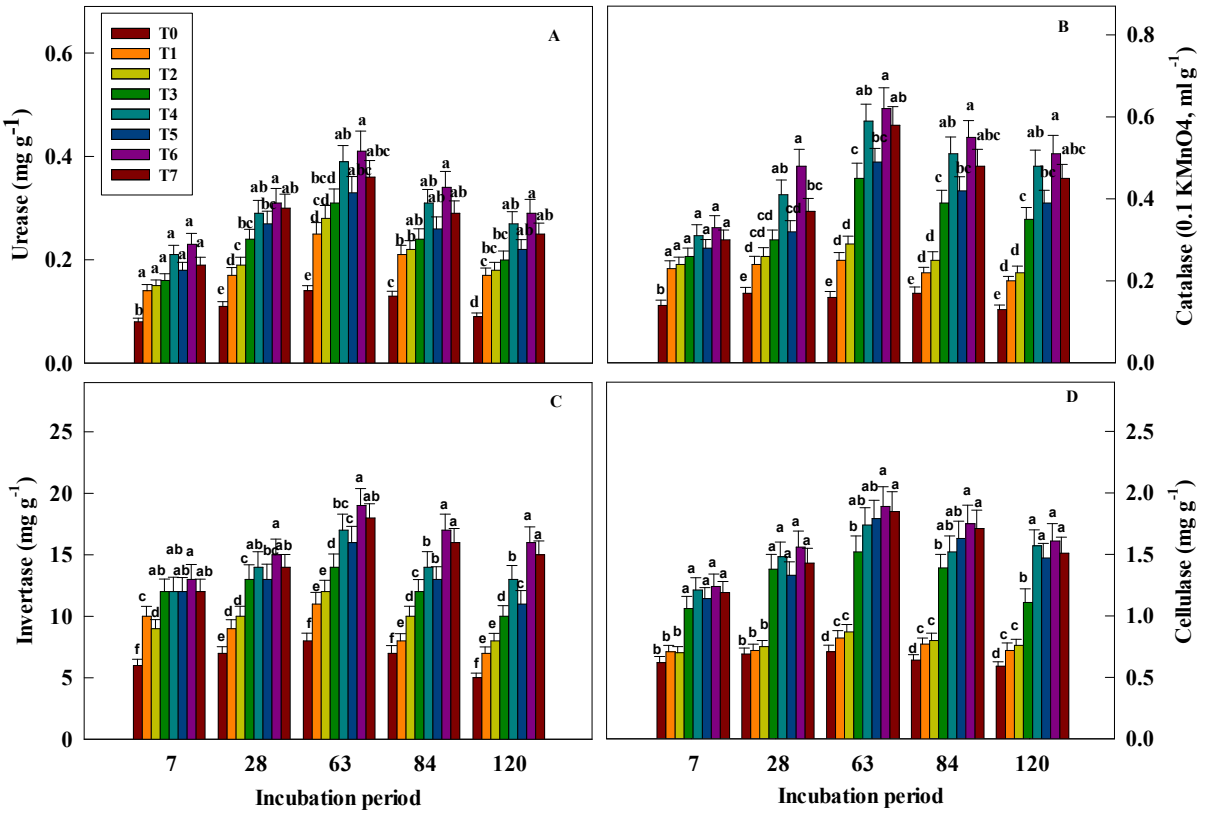

Figure 4. Soil enzyme activity in different treatments over the incubation period at different day intervals. (a) Urease, (b) Catalase, (c) Cellulase, and (d) Invertase

Note: Bars indicate standard errors (n = 4). Treatments with different letters are significantly different according to the LSD test ($p < 0.05$). T_0 = Control, T_1 = 100% CN, T_2 = 100% CN + MBF, T_3 = 75% CN + 25% PM + MBF, T_4 = 50% CN + 50% PM + MBF, T_5 = 25% CN+ 75% PM + MBF, T_6 = 100% PM + MBF, T_7 = 100% PM

Table 5. Correlation between the enzyme activities with SOC, MBN, MBC, and soil respiration rate at day 63 and day 120

Physiochemical and metabolic process of soil	Urease		Catalase		Invertase		Cellulase	
	63	120	63	120	63	120	63	120
SOC at day 63	0.758**	0.731**	0.882**	0.874**	0.898**	0.915**	0.916**	0.887**
SOC at day 120	0.722**	0.717**	0.829**	0.825**	0.875**	0.906**	0.841**	0.804**
MBN at day 63	0.711**	0.649**	0.848**	0.834**	0.866**	0.864**	0.793**	0.808**
MBN at day 120	0.640**	0.560**	0.784**	0.770**	0.838**	0.835**	0.746**	0.748**
MBC at day 63	0.792**	0.737**	0.914**	0.895**	0.941**	0.919**	0.917**	0.904**
MBC at day 120	0.885**	0.844**	0.955**	0.944**	0.986**	0.956**	0.948**	0.937**
Respiration at day 63	0.793**	0.752**	0.917**	0.906**	0.963**	0.962**	0.903**	0.888**
Respiration at day 120	0.773**	0.725**	0.887**	0.873**	0.952**	0.937**	0.895**	0.867**

Note: **Correlation is significant at the 0.01 level (2-tailed)

in all treatments, whereas other groups made up a small proportion in the contribution of total PLFA concentration. That means, on the one hand, the bacterial biomass was significantly higher in total PLFA concentration than the other groups among the treatments and over the incubation period. On the other hand, although the proportional concentrations of actinomycetes biomass, fungi biomass, and AMF biomass were low, they were significantly higher in the treatment where organic N was applied with microbial fertilizer. These concentrations increased with higher amounts of organic N. On day 63, the highest concentrations of actinomycetes, fungi, and AMF biomass were observed in treatment T_6, reaching 1.62, 1.86, and 0.60 nmol g^{-1}, respectively. Compared to the control treatment, these values were 2.00, 2.56, and 1.71 times higher, respectively (Figure 5 a-e).

PLFA concentration was shown to be a measure of microbial biomass (Pingree et al., 2022) and was used to examine the various soil communities of microbes fractions following the addition of various amendments to the soil. PLFAs are essential to the structure of any living organism's cell wall (Wen et al., 2023). Microorganisms that aid in the production of several microbiological biomarkers create diverse PLFAs. PLFA extraction provides general information on the soil microbial community (Zhang, 2023). According to some research, applying various fertilizers changed the microbial community's composition in the soil, which can be identified through PLFA patterns (Kaur et al., 2023; Li et al., 2023).

Soil microbial biomass decreases when CN is applied (Liu et al., 2023). A similar outcome was also observed in the present study, wherein the addition of CN (from a moderate to a high N proportion) reduced the total biomass of bacteria, actinomycetes, fungi, and AMF in the soil due to nutrient imbalances, soil acidification, and shifts in microbial competition, ultimately leading to a decrease in the total PLFA concentration. Notably, the control group had the lowest concentration of total PLFAs. However, increased application of PM promoted microbial population growth, likely due to the greater availability of substrates for microbial activity. Similar findings have been reported in previous studies, demonstrating that higher organic N application boosts the biomass of soil microorganisms (Pan et al., 2023).

The duration of incubation had a major impact on the soil's microbial community structure as well. As opposed to the latter stage, higher PLFA concentrations of microbial communities were seen on day 63 (Figure 5). The organic matter content of the soil may be connected with these fluctuations in PLFA biomass (Cheng et al., 2023). The mineralization of organic manure caused a drop in the soil's organic matter content during the incubation period, which also changed the makeup of the microbial community (Sun et al., 2023; Tan et al., 2023). Soil microbes primarily use microbial enzymes for metabolic processes, and various N sources significantly impact soil enzyme activities (Iqbal et al., 2023). Increasing CN content decreases enzyme activity, while moderate to higher organic N rates increase enzyme activity (Tong et al., 2023). PM addition increases microbial biomass and organic C deposition, enhancing urease enzyme activity. Increased clay content, higher organic matter, and microbial biomass also enhance soil urease enzyme activity. Overall, microbial activity and biochemical reaction intensity in soil are influenced by various N sources (Shen et al., 2022).

The soil's microbial community and enzyme activity are the basis of increased nutrient

availability and organic matter decomposition processes that support the establishment of environmentally friendly, sustainable agriculture. This experiment evaluated the availability of N in the soil, the microbial biomass with community structure responses, and the enzyme activity in response to various N sources and microbial fertilizer. The C pools, the composition of the microbial population, and the enzyme activity of the soil were all strongly impacted by the sources and amounts of N. The complete results showed that, in comparison to low PM rates, increasing PM rates enhanced the biological characteristics of soil and eventually changed the composition and community structure of soil microbial biomass. The makeup of the microbial population and the activity of soil enzymes are declining due to the application of higher levels of CN.

The highest level of N availability was found in treatment T_4, which concentrated on adding a medium level of organic and chemical N combination supplemented with MBF to create a more favorable environment for the development of microbes in the soil. Although the concentration of PLFAs and soil enzyme activities were found to be higher in treatment T_6, treatment T_4 had the highest level of N availability. The primary source of enzymes in the soil is the

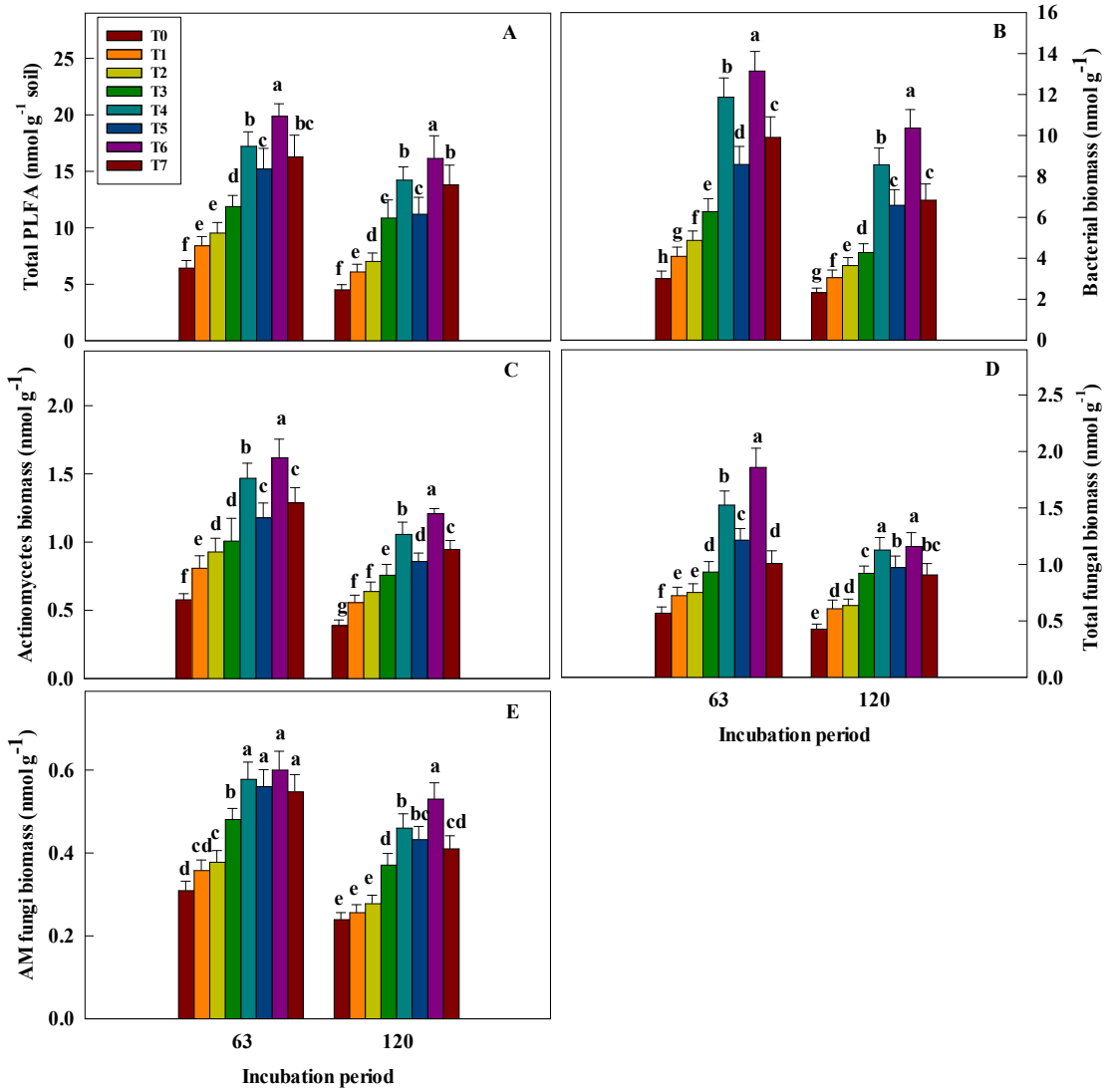

Figure 5. Phospholipid fatty acids concentration in soil under different treatments over the incubation period. (a) Total PLFA (total microbial biomass), (b) Bacterial biomass, (c) Actinomycetes biomass, (d) Total fungi biomass, (e) AMF biomass

Note: Bars indicate standard errors (n = 4). Treatments with different letters are significantly different according to the LSD test ($p < 0.05$). T_0 = Control, T_1 = 100% CN, T_2 = 100% CN + MBF, T_3 = 75% CN + 25% PM + MBF, T_4 = 50% CN + 50% PM + MBF, T_5 = 25% CN+ 75% PM + MBF, T_6 = 100% PM + MBF, T_7 = 100% PM

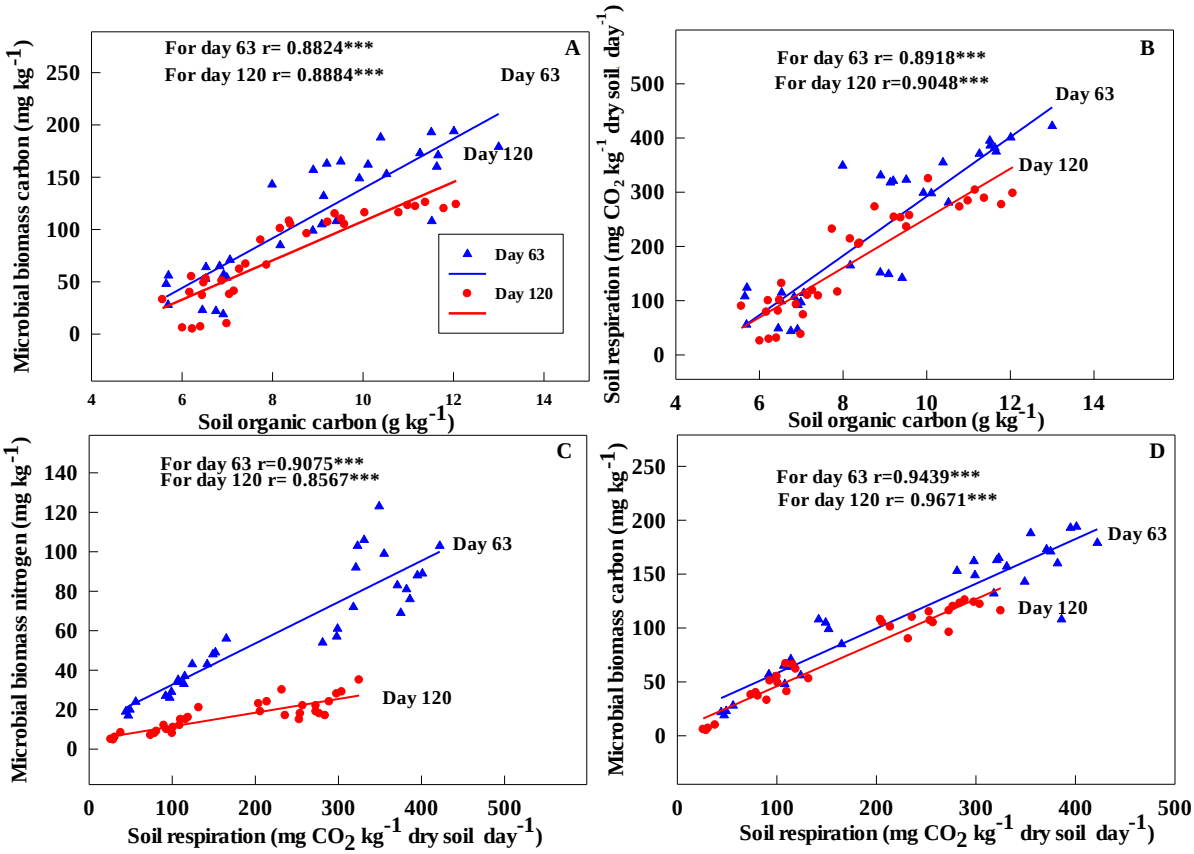

Figure 6. The relationship between (a) SOC and MBC, (b) SOC and soil respiration rate, (c) soil respiration rate and MBN, and (d) soil respiration rate and MBC

microbial biomass, which was increased by PM and microbial fertilizer. These factors also created a favorable environment for more microbial development in the soil. The large number of microorganisms in the soil is thought to increase the enzymatic activity of the soil, which is necessary for the metabolization of organic acids, amino acids, sugar, and other chemicals derived from soil organic matter. Thus, the application of 50% CN + 50% PM + MBF can improve the microbial structure and enzymatic activity in the soil, which is the main indicator of the good biological health of the soil.

Correlation between microbial biomass and physiochemical properties of soil

The correlation between microbial biomass and the soil's physicochemical and metabolic environment was analyzed to figure out how different treatments influence changes in microbial community structures. Strong positive correlations were found between MBC and SOC, soil respiration rate and SOC, MBN and soil respiration, and finally, the MBC and soil respiration rate at day 63 and day 120 (Figure 6). Additionally, four different enzyme activities with SOC, MBN, MBC, and soil respiration rate at day 63 and day 120 illustrated positively correlated at $p < 0.01$ (Table 5).

CONCLUSIONS

Applying PM alone or with CN improved soil properties by increasing organic matter and enhancing total and available N concentrations. PM can release significant mineral N, but its slow mineralization and low nutrient content limit its effectiveness as the primary nutrient source. This limitation can be overcome by combining PM with minimal CN and MBF. CN released N steadily but declined after 14 days, whereas treatment T_4 ensured continuous N release. The study suggests integrating organic and inorganic sources with MBF to enhance soil N availability and stability. Future studies should assess long-term field-scale applications to optimize fertilizer management for sustainable soil fertility and crop productivity.

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