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Research article

Optimized Initial Moisture Content Enhances Microbial Biomass and Nutrient Cycling During Aerobic Composting of Corn Straw and Cow Manure

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ABSTRACT

Optimizing the initial moisture content during composting is an important phenomenon for promoting microbial processes and nutrient dynamics in compost. Therefore, a study was carried out at Gansu Agricultural University, China (36°02'N, 104°25'E; 2400 m a.s.l.) to assess how an initially optimized moisture content (45% MC = MC_1, 55% MC = MC_2 and 65% MC = MC_3) affects the decomposition of corn straw and cow manure mixture in a bioreactor system during winter. The results obtained show that all treatments, except MC_1, entered the thermophilic phase (>50 °C) on day 3, with peak temperatures observed on day 7 for 55% MC (57.8 °C), day 9 for 45% MC (51.5 °C), and day 11 for 65% MC (56.7 °C). Despite the initial differences in moisture, the electrical conductivity (EC) values stabilized in the final phase of composting and were between 2.96 and 3.01 mS/cm for all treatments. The 65% MC outperformed the other treatments in microbial metrics. It had the highest microbial biomass carbon (MBC =254.81 mg/kg) and nitrogen (MBN = 26.38 mg/kg), corresponding to 6.7% and 6.9% increases over the 55% MC, and 14.8% and 26.4% over the 45% MC, respectively. The dissolved organic nitrogen (DON) content followed the trend 21.2 mg/g (MC_3) > 19.6 mg/g (MC_2) > 14.4 mg/g (MC_1), with MC_3 showing an increase of 47.2% and 8.2% over the MC_1 and MC_2, respectively. The total phosphorus (TP) content increased by 14.9%, 27.4%, and 35.2% for 45% MC, 55% MC, and 65% MC, respectively, compared to the baseline values. The extent of moisture loss was inversely proportional to the initial moisture content, with MC_1 (44%) showing the highest losses, while MC_3 showed the lowest (23.4%). These results suggest that optimizing the initial moisture content of corn straw and cow manure mixture to 65% significantly improved the stimulation of microbial biomass, nutrient retention and overall quality of the compost.

Key words: *composting, corn straw, cow manure, moisture content, microbial biomass, nutrient dynamics, compost*

Abbreviations:
EC – electrical conductivity; DON – Dissolved organic nitrogen; MBC – microbial biomass carbon; MBN – microbial biomass nitrogen; MC – moisture content; TP – total phosphorus

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INTRODUCTION

The shift from indigenous agricultural practices, driven by population growth and the increasing preference for nutritious food has exacerbated the accumulation of untapped solid waste in China. Notably, agriculture generates approximately 80.5 million tons (Mt) of crop residues and 261 Mt of animal manure each year (Cao et al., 2023), with livestock manure and crop straw constituting the bulk waste stream (Jia et al., 2018). In many farming communities, residues from maize, rice, wheat, potato, beans etc. are regarded as waste and are commonly burned after harvest as an expedient and cost-prohibitive method to control pests and diseases, eliminates weed seeds, and prepare cropping fields for the next planting season (Li et al., 2016; Cui et al., 2021). This human-initiated practice significantly contributes to seasonal air pollution owning to the emission of particulate matter ($PM_{2.5}$), carbonaceous soot, nitrogen oxides (NO_X), sulphur dioxide (SO_2), and polycyclic aromatic hydrocarbons (PAHs) (Zhou et al., 2017). Despite the abundance of organic waste resources, China remains the dominant global consumer of synthetic fertilizers (Hu et al., 2023), allocating nearly 30% of its agricultural expenditure to the input (Huang et al., 2008). Some common waste management practices, such as incineration, landfilling, and open burning, are very harmful to the environment (Cheng and Hu, 2010; Ma and Liu, 2019), and different practices are necessary to mitigate the consequences of the practices (Figure 1). Corn straw and livestock manure contain valuable nutrients (carbon, nitrogen, phosphorus, potassium, and other minerals) that can be transformed aerobically into compost for crop production (Jia et al., 2018). While corn straw is predominantly carbon-rich, cow manure is nitrogen-rich, promoting a balanced carbon-to-nitrogen ratio that enhances microbial activity. According to Prasad et al. (2020), the bioconversion of biological resources into compost can replace synthetic fertilizers or complement a nutrient-rich fertilizer mix for sustainable cropping system while promoting environmental and economic sustainability.

Microbial biomass represents a readily decomposable fraction of organic matter and serves as a sink for essential nutrients required by plants (Bhuyan et al., 2013; Li et al., 2018). In spite of its limited physical size, microbial biomass consists of a wide array of microorganisms (bacteria, actinomycetes, fungi, algae, protozoa etc) that collectively enhance compost maturation and stability (Das et al., 2023). Through decomposition and synthesis of microbial biomass, microorganisms actively participate in the biocycling of carbon (C), nitrogen (N), phosphorus (P), sulphur (S), and other key minerals. This dynamic participation makes microorganisms the metabolic engine and integral sink for nutrient turnover and assimilation in compost (Zak et al., 2003; Rotbart, 2019; Wang et al., 2023). Although composting facilitates the mineralization of microbial biomass, the natural resistance of lignocellulosic materials to decomposition often necessitates the use of synthetic, microbial and mineral additives along with pretreatments including thermal hydrolysis, steam explosion or hydrogen peroxide application to hasten the process (Bhatia et al., 2020). However, these additives and treatments adversely affect microbial richness, diversity, and robustness, leading to incomplete fermentation and reduced compost quality (Wang et al., 2018; Yuan et al., 2020). In addition, these treatments are cost-prohibitive and often impractical under resource-poor conditions (Ayilara et al., 2020). Our previous study established water as a universal and inexpensive solvent, capable of reducing the inherent resistance of lignocellulosic materials when efficiently regulated (Ghanney et al., 2021; Ghanney et al., 2023). A well-regulated moisture scenario can minimize nutrient losses through leaching, thus creating a favorable environment for microbial proliferation and decomposition during composting. Therefore, this study is designed to (i) unravel how water impacts microbial biomass development, (ii) determine the moisture condition optimal for compost maturation and stability, and (iii) establish an optimal moisture content that best stimulates microbial biomass development and nutrient retention during composting.

MATERIAL AND METHODS

Area of Study and Organic Resources

The experiment on moisture optimization for composting was conducted during winter at Gansu Agricultural University, Lanzhou, China (36.0904° N, 103.6946° E). A total of nine identical bioreactors were set up and precisely regulated at a relative humidity of 60% and a temperature of 16.2 ± 0.23°C. The plant (corn straw) and animal resources (cow manure) for composting were gathered from the fecal matter deposition site at Lanzhou Yili Dairy Company Limited and the maize-growing fields in Yuzhong County, China. These areas were chosen to draw farmers' attention in Gansu Province to the critical role of organic resources in promoting the circular economy. The exposure of these resources to routine burning has significant ecological and environmental consequences detrimental to human health (Figure 1).

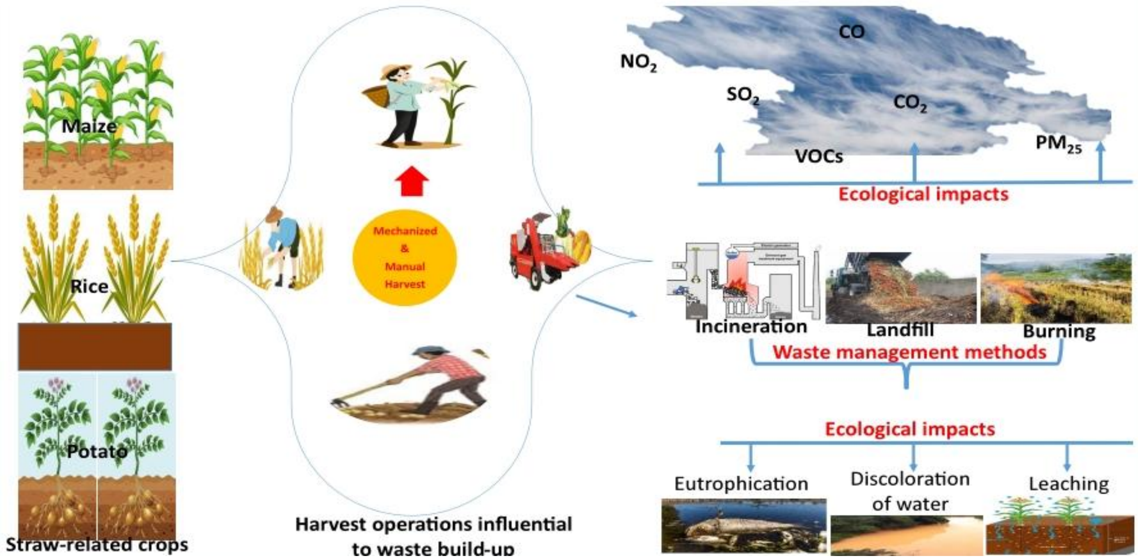

Figure 1. Graphical representation of the ecological and environmental consequences of waste management practices

Pre-Conditioning of Organic Substrates and Reactor-Based Composting Procedure

Before composting, the corn straw and cow manure were spread out under natural aeration condition to dry until no further changes in mass were observed. Subsequently, the straw was shredded to maximize surface exposure for microbial interactions. To prepare the compost substrate, 15 kg of cow manure and 4 kg of corn straw were measured and placed in a commercial mixing machine for thorough mixing. As the mixer revolved, a measured amount of distilled water was applied to the organic materials in calculated quantities to bring their initial moisture levels to 45% = MC_1, 55% = MC_2, and 65% = MC_3. Each moisture regime was carried out in triplicate to ensure reliability and reproducibility of results. The prepared feedstock was promptly placed in the bioreactors (Figure 2) to prevent drying, and subjected to an intermittent airflow of 0.4 L min^{-1} (10 minutes on, 10 minutes off) to maintain adequate oxygen availability. The composting process was deemed physically complete after 31 days, when the temperature of the decomposing materials leveled off, approaching the surrounding ambient temperature (16.2 ± 0.23 ^oC). In brief, the bioreactors were insulated with double-layer cotton wool enclosed in a stainless-steel casing. A valve positioned at the bottom of the reactor facilitated the introduction of oxygen into the decomposing substrate. Two outlet valves were installed on the top of the reactor, one for gas sampling and the other to hold the thermometer securely. To achieve consistent air distribution in the reactor system, a 5 cm-thick sealing film was positioned above the inlet valve (Figure 2). The programmable control unit (PCU) of the bioreactor ensured operational functions such as agitation, temperature and gas regulation, and data logging.

Figure 2. Graphical representation of the bioreactor components and the composting process: (a) switch; (b) programmable control unit (PCU); (c) stand, (d) gas cylinder; (e) gas supply line; (f) automatic point of oxygen regulation, (g) inlet valve; (h) feedstock; (i) reactor headspace; (j) insulator; (k) outlet valve; (l) point of gas collection; (m) rotator; (n) point of thermometer fixation; (o) reactor lid; (p) thermometer; and (q) seal liner

Experimental Setup and Sampling Approach

The study investigated three moisture levels (45% = MC_1, 55% = MC_2, and 65% = MC_3) using a completely randomized design (CRD) with three replicates (Figure 3). Samples were taken in triplicate at two-day intervals from the upper, middle, and bottom sections of the reactor. In addition, daily measurements were taken for both temperature and pH, whereas moisture content was measured every five days. From each section of the reactor, 100 g portion of fresh solid material was taken and thoroughly mixed according to the respective moisture treatments. The mixed samples were divided into two portions, with one portion exposed to natural air-drying and the other stored at −4 °C in the refrigerator. The air-dried samples were pulverized (Retsch ZM_{200}, Germany) and passed through a 0.25 mm sieve for microbial biomass and nutrient analysis. In addition, the air-dried samples were analyzed for microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), pH, and electrical conductivity (EC), while the freshly collected samples were assessed for dissolved organic carbon (DOC) and nitrogen (DON), moisture content (MC), total nitrogen (TN), total organic carbon (TOC), total phosphorus (TP), total potassium (TK), and germination index (GI).

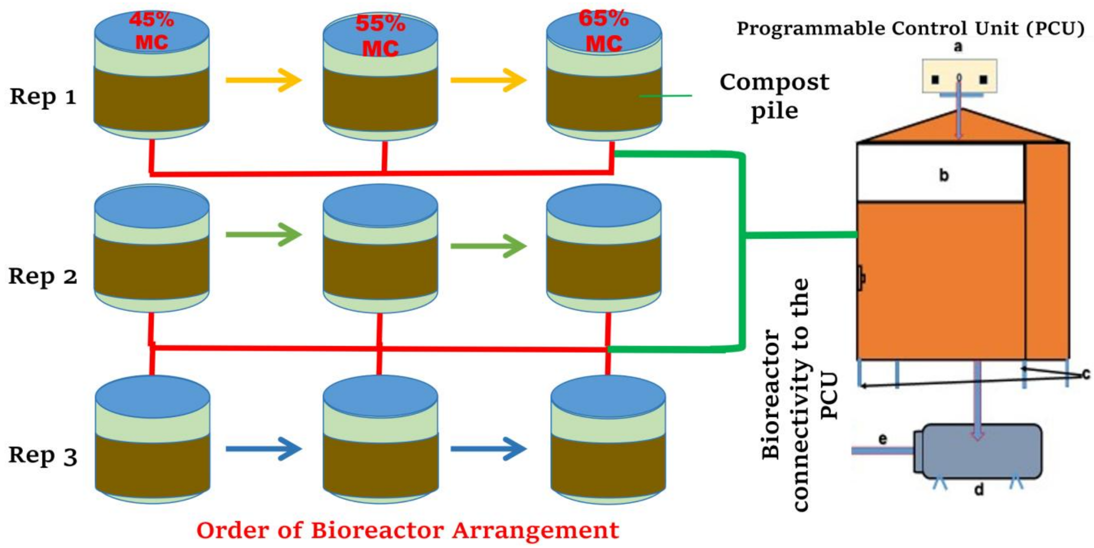

Figure 3. The bioreactor setup for evaluating the microbial metrics and nutrient dynamics during composting: (a) switch; (b) programmable control unit (PCU); (c) stand, (d) gas cylinder; (e) gas supply line

The temperature of the compost and ambient environment was recorded hourly and tabulated into daily average temperatures on the PCU, whereas electrical conductivity (EC) and pH were measured following the protocol outlined by Ren et al. (2019). By measuring these parameters, dry representative samples were diluted with deionized water at 1:10 weight-to-volume ratio (w/v) and agitated for 30 minutes. The MP_{521} pH/EC electrode (Shanghai, China) was placed in the solution to record the pH and electrical conductivity after allowing the mixture to settle for 30 minutes. The microbial biomass in the finished product was quantified through the chloroform fumigation extraction technique described by Vance et al. (1987). On this, 5 g of fresh compost was placed in a desiccator and exposed to trichloromethane ($CHCl_3$) for 16 hours to lyse microbial cells. The samples were extracted immediately after fumigation with 0.5 M potassium sulfate (K_2SO_4) to release microbial carbon and nitrogen into the solution. The extracts were then filtered and analyzed for carbon and nitrogen concentrations using the TOC analyzer and a Kjeldahl digestion method, respectively. The non-fumigated samples were processed simultaneously to account for background organic carbon and nitrogen fractions. The estimation of microbial biomass parameters, specifically microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), was performed by calculating the difference between fumigated and non-fumigated samples and applying an appropriate conversion factor. Their calculations were performed using the following equations:

$$MBC\ (mg\ C\ kg^{-1}) = 2.22 * (MB\ fumigated\ -\ MB\ non\text{-}fumigated) \quad [1]$$
$$MBC\ (mg\ N\ kg^{-1}) = 0.54 * (NB\ fumigated\ -\ NB\ non\text{-}fumigated) \quad [2]$$

where
MBC = microbial biomass carbon
MB = organic carbon extracted from the fumigated and non-fumigated samples
2.22 = extraction efficiency constant for carbon
MBN = microbial biomass nitrogen
NB = nitrogen extracted from the fumigated and non-fumigated samples, and
0.54 = extraction efficiency constant for nitrogen.

The extraction procedure of dissolved organic carbon (DOC) followed the methodology of Chefetz et al. (1998). Subsequently, 10 g of the compost sample was precisely weighed into a glass bottle and 100 mL of distilled water was added. The pH of the solution was brought to neutrality (7.0) using NaOH, after which it was shaken at 150 rpm for 24 hours under room temperature and centrifuged at 5000 rpm for 20 minutes to separate the supernatant from the solid residue. The supernatant was subjected to a second centrifugation to obtain a clear solution, after which the dissolved organic carbon was quantified using high-temperature combustion. The extraction procedure outlined by Gigliotti et al. (2002) was then employed for determining the dissolved organic matter (DOM). In this process, DOM was extracted from wet samples using distilled water at a 1:10 (w/v) ratio and subsequently centrifuged at 6000 rpm for 15 minutes at 4 °C. The supernatant obtained was passed through a 0.45 µm membrane filter to obtain a clarified extract for subsequent analysis. The germination index (GI) of the compost was determined using the method of Zucconi et al. (1981). A fresh compost sample (10 g) was mixed with 100 mL distilled water (1:10, w/v) and shaken using a rotary shaker at 180 rpm for 30 minutes at 25 °C to extract water-soluble compounds. The suspension was allowed to settle and then filtered through Whatman No. 1 filter paper to obtain a clear aqueous extract. Afterwards, 5 mL of each extract was transferred into a double-sterilized Petri dish lined with two layers of Whatman No. 42 filter paper. Twenty seeds of Chinese cabbage (*Brassica rapa subsp. chinensis*) were evenly placed in each dish and replicated three times per treatment. The Petri dishes were incubated in the dark at 25 °C for 5 days, after which the number of germinated seeds was recorded and root lengths measured. The germination index (GI) was calculated as follows:

$$GI\ (\%) = \frac{\text{Seed germination in compost extract} \times \text{Root length in compost extract}}{\text{Seed germination in control extract} \times \text{Root length in control extract}} \times 100$$

Statistical Analysis

All measurements were performed in triplicate and the resulting data organized and summarized using Microsoft Excel (version 2016). Treatment effects were evaluated by the one-way ANOVA using IBM SPSS (version 22). Treatment means were separated at the 5% probability level, and figures were generated using GraphPad Prism. Results are presented as mean ± standard error (n = 3).

RESULTS AND DISCUSSION

Dynamics in Temperature, pH and Electrical Conductivity

The temperature (Figure 4a) of the compost heap rose gradually over the first eight days before falling again. This shows that the biodegradation process was slow due to manual aeration, which was continuously carried out for 1 week. As noted by Cai et al. (2023), turning the pile stimulates microbial activities, which subsequently promotes the decomposition of organic matter, thereby accelerating biodegradation. However, the frequency in turning could have disrupted and destabilized the microorganisms, forcing most species to spend additional time to re-establish and form robust communities for effective heat activation and stabilization (Cook et al., 2015). All treatments, except 45% MC, entered the thermophilic phase (>50 °C) on day 3, but peaked on different periods: day 7 for 55% MC (57.8 °C), day 9 for 45% MC (51.5 °C) and day 11 for 65% MC (56.7 °C). The variation in peak timing likely reflects the impact of the initial moisture on microbial activity, oxygen availability, and the rate of organic matter decomposition. The 45% MC treatment may have limited microbial metabolism and delayed heat buildup, while the 65% MC may have promoted rapid microbial growth and greater thermal stability.

The three moisture levels showed significant differences in pH ($p < 0.001$) despite their similar trends (Figure 4b). Within the initial five days of composting, the pH rose across all treatments, reaching a value of 9.0 > 8.9 > 8.7 for MC_1, MC_2, and MC_3, respectively. The observed rise can be attributed to the decomposition of organic nitrogen compounds (proteins and amino acids) which releases ammonia (NH_3) into the compost media. As reported, alkaline substances are produced during substrate decomposition which increases pH (Bai et al., 2020). However, after the fifth day, pH values steadily declined to the completion period, showing final values of 8.5 for MC_1 and 8.4 for both MC_2 and MC_3. This reduction is likely associated with CO_2 emissions, organic matter decomposition, and nitrification processes reported in our previous study (Ghanney et al., 2021). The interaction between CO_2 and water produces carbonic acid (H_2CO_3) whose dissociation releases hydrogen ions (H^+) which can easily lower the pH of the compost (Yuan et al., 2016). In addition, the breakdown of organic matter produces organic acids (acetic acid [CH_3COOH], lactic acid [$C_3H_6O_3$], and citric acid [$C_6H_8O_7$]) whose dissociation releases hydrogen ions which lower the pH of the compost (Wei et al., 2018). The variation in pH from day 5 to day 31 was lowest for the 65% MC (0.2) compared to the 55% (0.5) and 45% (0.5) MCs. This decrease could be due to 65% MC allowing for better aeration and oxygenation to support active microbial metabolism, but not high enough to impede airflow or cause an anaerobic condition. Despite the decline in pH, all treatments remained alkaline at the final stage of composting, confirming the compost stability for acidic soil remediation.

The values derived on electrical conductivity (EC) showed a clear trend, with the initial value decreasing from 3.08 mS/cm to 2.78 mS/cm on day 5 (Figure 4c). The observed decrease could be due to early microbial uptake of soluble ions and redistribution of salts within the compost matrix (Said-Pullicino et al., 2007; Shin and Son, 2015). When the microbial population is active in the early stage of composting, it assimilates available nutrients such as ammonium, potassium, and phosphates, which temporarily lowers the concentration of free ions in the compost extract and thus, the pH value. Despite variations in initial moisture content, final EC values ranged from 2.96 to 3.01 mS/cm, indicating that soluble salts were effectively diluted across all treatments. This also suggests that each moisture level enabled redistribution and stabilization of the salts within the compost matrix. In addition, the uniformity could indicate the prolonged microbial mineralization and gradual stabilization of the compost matrix, with nutrients either assimilated into the microbial biomass or bound in more stable organic forms. Nevertheless, the uniformity in EC values emphasizes the ability of the moisture treatments to reduce salt accumulation while ensuring that the final product remains suitable for application on saline or sodic soils (Gondek et al., 2020).

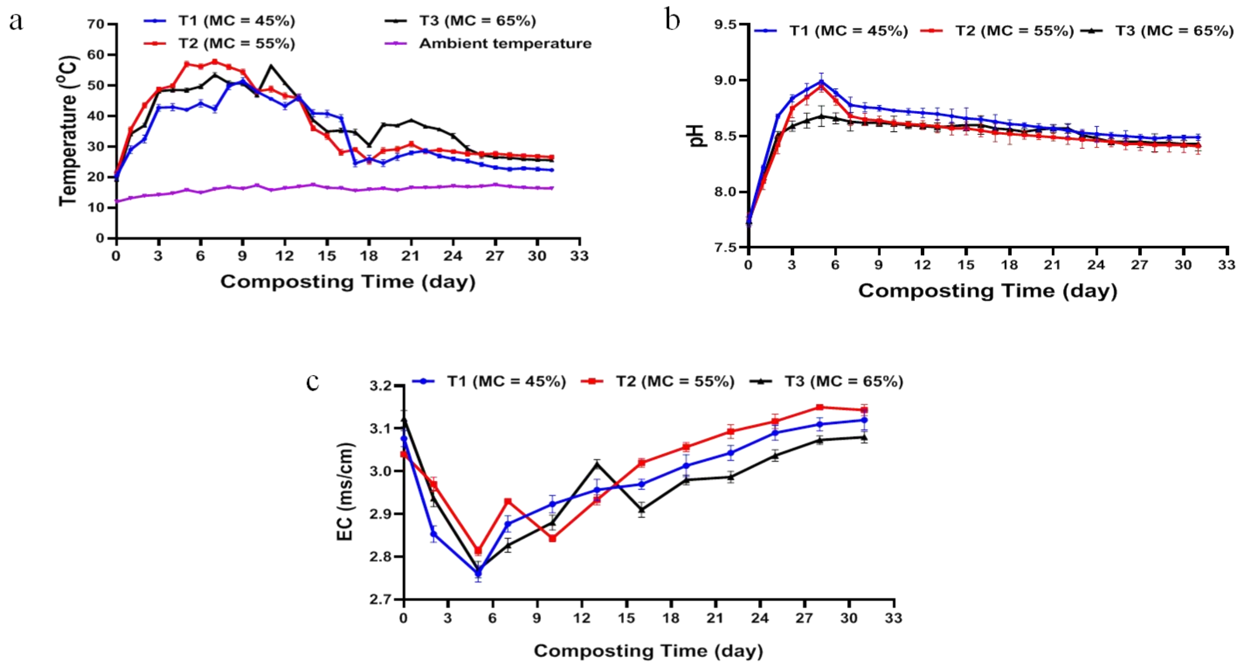

Figure 4. Temporal dynamics in temperature (a), pH (b), and electrical conductivity (c) as affected by initial moisture optimization. Error bars indicate ± standard error of means (n = 3)

Changes in Microbial Biomass

Moisture levels had a significant effect ($p < 0.001$) on microbial biomass carbon (MBC), with the highest accumulation observed in compost heaps initiated at 65% MC (Figure 5a). Across all moisture treatments, MBC increased from an initial value of 55.3 mg kg^{-1} to a maximum of 332.5 mg kg^{-1} during the composting period at Gansu Agricultural University. The amount of MBC produced by MC_3 (254.81 mg/kg) increased by 16.0 mg/kg (6.7%) compared to MC_2 (238.81 mg/kg) and by 32.8 mg/kg (14.77%) compared to MC_1 (222.01 mg/g) (Figure 5b). The highest MBC observed under the 65% moisture content could be that the treatment created optimal conditions for the microbiota to reach and hydrolyze the corn straw as an energy source for microbial biomass synthesis. As an optimal energy source (Iqbal et al., 2015; Lin et al., 2018), corn straw might have actively attracted more microorganisms for decomposition and humification, thereby increasing MBC. In addition, the 65% MC may have hindered the mobility of heavy metals, thereby minimizing their inhibitory effect on microbial activity (Shen et al., 2016).

As shown in Figure 5c and 5d, the microbial biomass nitrogen (MBN) showed a trend similar to the microbial biomass carbon (MBC), indicating a progressive increase in nitrogen mineralization. This is certain as nitrogen is initially immobilized during the decomposition of the organic substrate. However, as the composting process progresses, microbial turnover and enzymatic activity enhance the release of mineral nitrogen (ammonium (NH_4^+) and nitrate (NO_3^-) into the compost matrix, thereby increasing the buildup of nitrogen (Zhou et al., 2014; Iqbal et al., 2015). In general, the amount of MBN was relatively low compared to MBC and differed significantly between moisture treatments ($p < 0.003$) and composting periods ($p < 0.001$). This discrepancy was due to the fact that the compost mixture (corn straw and cow manure) contained more carbon than nitrogen. Since microorganisms generally have a greater need for carbon than for nitrogen to support growth and metabolism, this may have led to greater accumulation of MBC than MBN. In addition, nitrogen is often immobilized during the early phase of decomposition and can be lost through processes such as ammonification, nitrification, and volatilization. This could partly explain the low MBN production. Overall, MC_3 had the highest MBN (26.38 mg/kg), exceeding MC_2 (24.68 mg/kg) by 1.7 mg/g (6.9%) and MC_1 by 26.4% (5.5 mg/kg).

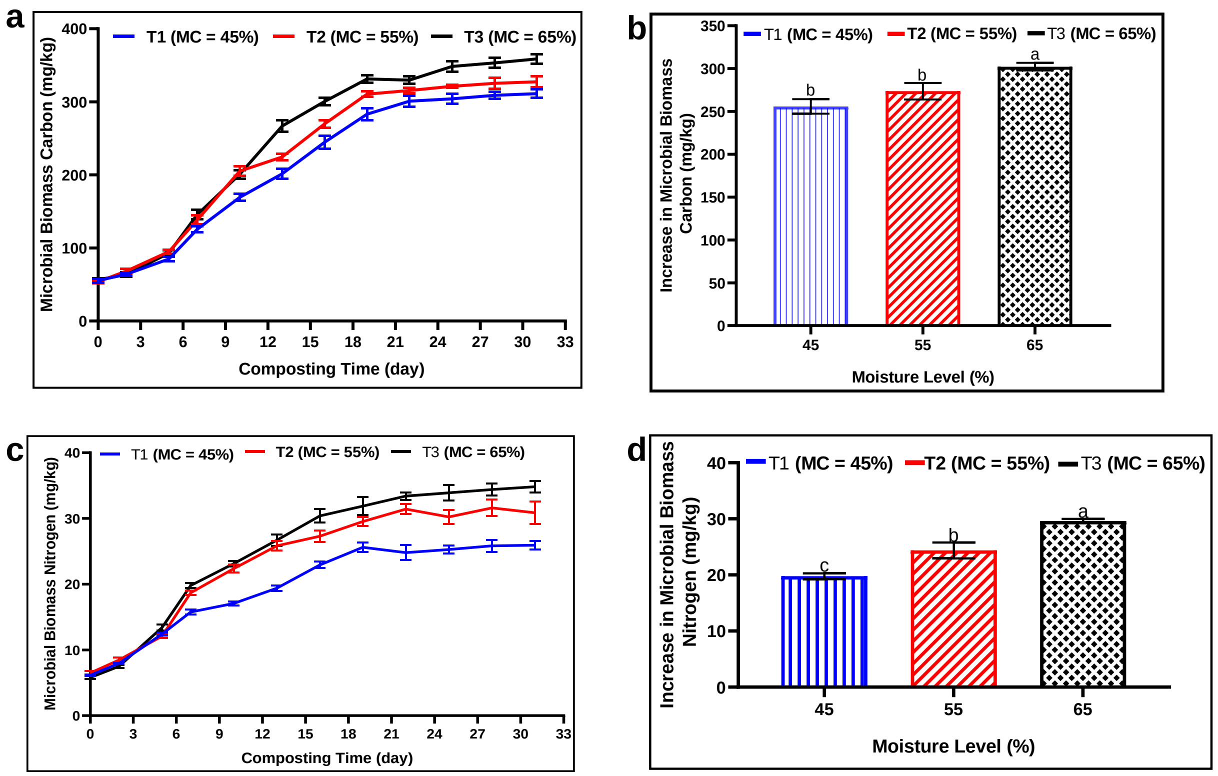

Figure 5. Temporal dynamics of microbial biomass carbon (a and b) and microbial biomass nitrogen (c and d) as affected by initial moisture optimization during composting. Error bars indicate ± standard error of means (n = 3)

Dissolved Organic Carbon and Nitrogen Variations

The dissolved organic carbon (DOC) content increased from 16.83 mg/g at the start of composting to a maximum of 39.05 mg/g on day 7, before decreasing to 8.4 mg/g at the end of composting (31 days) (Figure 6a). The increase during this period may be due to early microbial adaptation and proliferation, which could have stimulated the release of soluble organic compounds (Zhou et al., 2024). In addition, the early temperature rise could have also promoted the phenomenon through the solubilization and degradation of soluble carbon compounds. Conversely, the decrease in DOC could result from microbial consumption of available biochemicals (sugars, amino acids, and organic molecules) within the compost matrix (Zmora-Nahum et al., 2005). The difference in DOC between the start (MC_1 = 16.06 mg/g, MC_2 = 16.82 mg/g, MC_3 = 16.27 mg/g) and peak period (MC_1 = 34.01 mg/g, MC2 = 39.42 mg/g, MC3 = 43.73 mg/g) of composting showed the trend: 17.9 mg/g > 22.6 mg/g > 27.4 mg/g for MC_1, MC_2, and MC_3 respectively (Figure 6a). The greater increased observed in the 65% MC may be attributed to the enhanced breakdown of complex organic matter into low molecular-weight, water-soluble compounds by the treatment. Since all moisture levels are within an optimal range, they may have facilitated the diffusion and transport of soluble organic compounds from the solid phase into the composting solution, thereby contributing to DOC buildup. At the end of composting, the DOC concentration showed a decreasing trend across all treatments: 8.9 mg/g > 8.4 mg/g > 7.8 mg/g for MC_1, MC_2, and MC_3, respectively, indicating that the compost had matured and stabilized. As indicated by Wang et al. (2018), compost with DOC content below 10 mg/g is considered sufficiently mature and free from phytotoxicity.

Figure 6c and 6d show an increase in dissolved organic nitrogen (DON), with concentrations ranging from 3.4 mg/g to 27.8 mg/g across the composting cycle. This upward trend could originate from microbial mineralization of organic nitrogen compounds, in which microorganisms breakdown complex nitrogenous materials into simpler and soluble forms. As Blesh and Drinkwater (2014) established, microbially driven mineralization processes enhance DON content in compost as microorganisms actively decompose organic substrate and release nitrogen into the dissolved phase. At the initial stage, the DON content was highest in MC_2 and lowest in MC_1. This variation could be due to MC_2 being sufficient to dissolve and mobilize amino acids, peptides, and low-molecular-weight organic nitrogen compounds for DON formation. According to Zhang et al. (2020), sufficient moisture improves the substrates structure for microbial degradation and nutrient release. In contrast, the lower DON observed under MC_3 may be that the higher moisture treatment promoted rapid microbial assimilation of soluble nitrogen compounds and possible dilution effects, thereby limiting DON accumulation at the initial stage. A significant difference in DON ($p < 0.004$) content was observed across the moisture treatments (Figure 6 c and d). However, the highest DON content was observed at 65% MC, highlighting the importance of adequate moisture for microbial efficiency. This outcome is congruent with the findings of Kim et al. (2015), who suggested that higher moisture content softens organic substrates to improve biodegradation and nutrient release. In general, DON concentration was 14.4 mg/g, 19.6 mg/g, and 21.2 mg/g for MC_1, MC_2, and MC_3 respectively, with MC_3 showing an increase of 6.8 mg/g (47.2%) over MC_1 and 1.6 mg/g (8.16%) over MC_2.

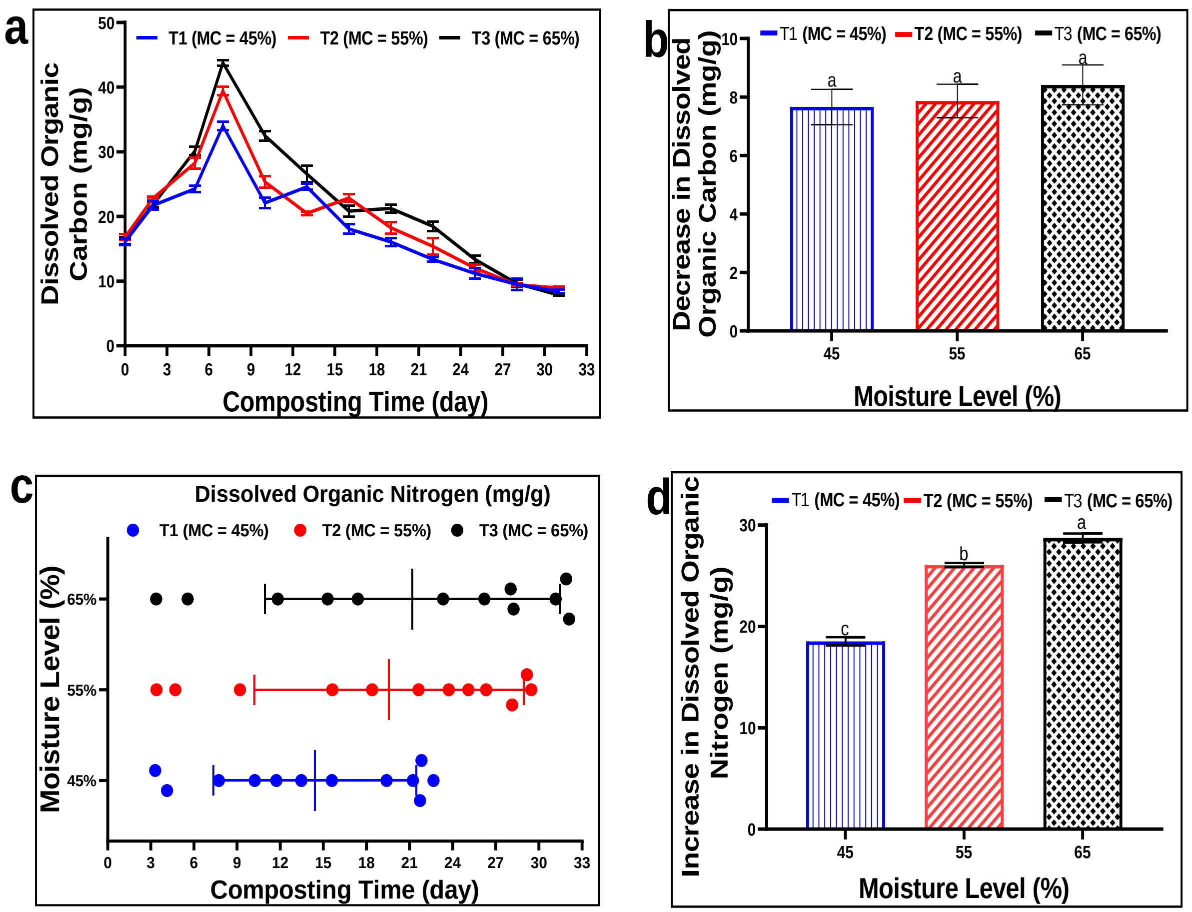

Figure 6. Temporal dynamics of dissolved organic carbon (a and b) and dissolved organic nitrogen (c and d) as affected by initial moisture optimization during composting. Error bars indicate ± standard error of means (n = 3)

MBC/MBN and DOC/DON Ratios

Figure 7a shows an increasing trend in the MBC/MBN ratio for all treatments. This upward trend indicates increased carbon assimilation comparable to that of nitrogen. However, a decrease was observed within the first five days, which could be due to nitrogen immobilization resulting from microbial uptake of labile substrates during the mesophilic phase. Among the moisture levels, the 45% moisture treatment resulted in the highest ratio, indicating an enhanced carbon storage (less carbon decomposition) and limited nitrogen mineralization during the process. In addition, the 45% moisture level may have led to a drier condition, suppressing nitrogen-transforming microorganisms and thus, reducing nitrification. Pile formulation at 45% MC showed increases of 0.8 mg/g (7.8%) and 0.9 mg/g (8.9%) comparable to formulations at 65% and 55% MCs, respectively.

The DOC/DON ratio decreased from 4.9 to 0.4 throughout the composting period, with the most rapid decrease observed in the 65% MC (Figure 7b). This pronounced decrease indicates that nitrogen mineralization exceeded carbon solubilization (Blesh and Drinkwater, 2014), particularly in the 65% MC. The increased nitrogen turnover under the 65% MC may have been sufficient to soften the compost matrix, leading to microbial colonization and enzymatic degradation (Kim et al., 2015). All treatments showed a higher DOC/DON ratio during the thermophilic phase, reflecting increased microbial activity and organic matter degradation. On average, the DOC/DON ratio across all treatments followed the trend: MC_1 (2.1) > MC_2 (1.8) > MC_3 (1.7), indicating that higher moisture content promotes greater accumulation of dissolved organic carbon than nitrogen.

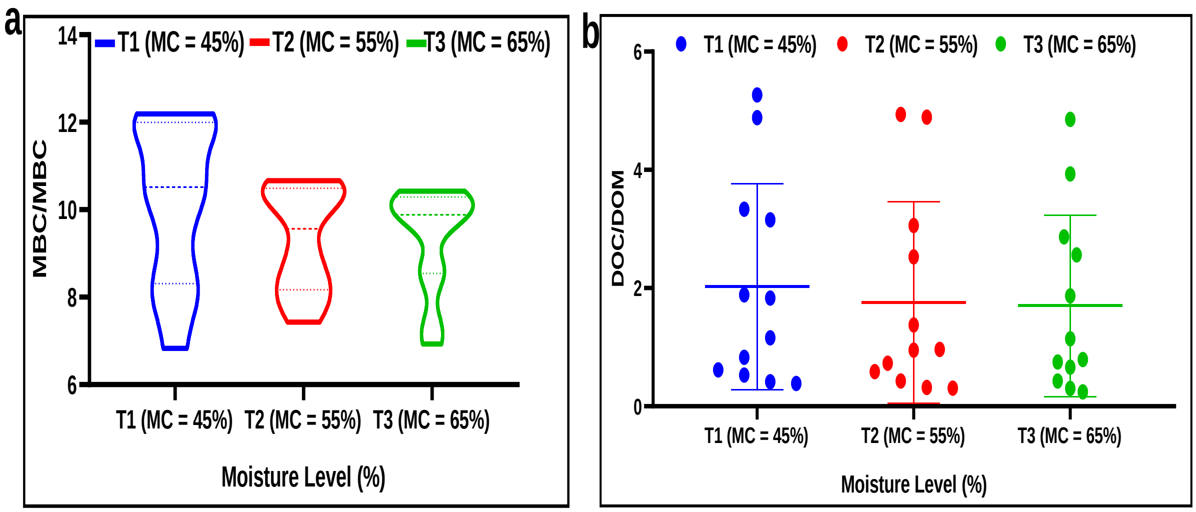

Figure 7. Temporal dynamics in MBC/MBN (a) and DOC/DON (b) ratios as affected by initial moisture optimization during composting. Error bars indicate ± standard error of means (n = 3)

Variations in Nitrogen and Organic Carbon Fraction

The nitrogen content of the substrate decreased in the first two days, followed by a steady rise thereafter (Figure 8a and b). The decline is linked to ammonia volatilization, which was high during the thermal-active phase of composting. As reported in our earlier investigation (Ghanney et al., 2021), the thermophilic phase increased pH and temperature, which favored the NH_3 emission and thus decreased TN. As highlighted by Li et al. (2021), ammonia volatilization is a major route of nitrogen diminution during composting. Additionally, TN increased consistently across all treatments, with the 65% MC showing an increase of approximately 5.7 mg/g (35.6%) compared with the 55% and 45% MCs. This increase could be due to the treatment (65% MC) providing a suitable environment that supports the nitrification performance of microorganisms for the conversion of ammonium to nitrate. According to Huang et al. (2004), this phenomenon not only stabilizes the nitrogen in non-volatile forms, but also minimizes the ammonia (NH_3) loss, as sufficient moisture promotes nitrification and supports methane oxidation. At compost maturity, TN content showed 33.3% (5.3 mg/g) increase compared to the starting period. This increase is consistent with the observation of Santos et al. (2016), who reported that nitrogen mineralization increases with increasing composting duration. A long duration promotes extensive decomposition of organic nitrogen into plant-available forms. In contrast, the decrease was attributed to elevated pH which favored the loss of ammonia in the early stages (Afonso et al., 2021). Despite the initial losses, TN concentrations above 16 mg/g were obtained upon compost maturation across all treatments. This shows that the compost product contained sufficient N for plant uptake and is unlikely to result in net nitrogen immobilization in soils.

Over the compositing period, total organic carbon (TOC) content gradually decreased and differed markedly ($p < 0.03$) among the moisture levels, with fluctuations mainly observed under the 45% and 65% moisture conditions (Figure 8c and d). The observed decrease over time can be traced to microbial involvement in the breakdown of organic matter, where carbon is metabolized and released as carbon dioxide (CO_2), particularly under higher moisture conditions. TOC decreased from 654.0 mg/g to 513.0 mg/g, a 21.36% decrease. This moderate loss indicates that decomposition was efficient and the extent of organic matter breakdown remained balanced between the moisture treatments. The 65% MC treatment had the lowest TOC values while the 45% MC had the highest. The reduction observed with the 65% MC may be attributed to oversaturation, which limits oxygen diffusion within the compost matrix, slowing aerobic decomposition.

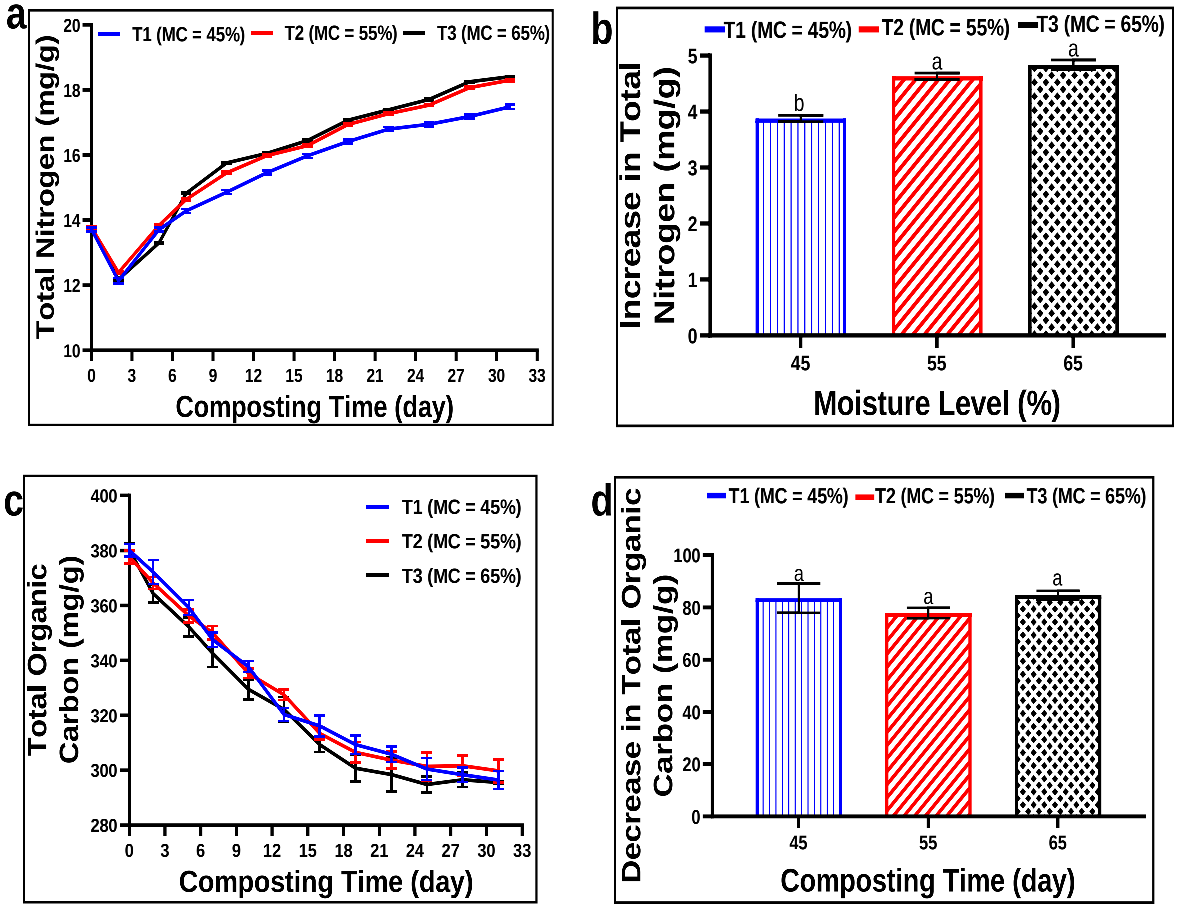

Figure 8. Temporal dynamics of total nitrogen (a and b) and total organic carbon (c and d) as affected by initial moisture optimization during composting. Error bars indicate ± standard error of means (n = 3)

Variations in Phosphorus and Potassium

The initial total phosphorus (TP) content was 6.1 mg/g for all treatments. However, the concentration of TP decreased slightly in MC_1 (45%) and MC_3 (65%), but increased in MC_2 (55%) treatment on the second day of composting. This occurrence may reflect early microbial adaptation and phosphorus immobilization due to the different microbial activities at the beginning of decomposition. From day 3 onwards, TP levels increased for all treatments and ranged from 6.3 mg/g to 8.3 mg/g (Figure 9a and b). This increase is likely due to the progressive decomposition of organic matter and microbial turnover, which releases assimilated phosphorus back into the compost matrix as the decomposition progresses. Statistically, TP differed significantly ($p < 0.01$) across the treatments, with MC_1, MC_2, and MC_3 at 6.9 mg/g, 7.6 mg/g, and 7.9 mg/g, respectively (Figure 9a and b). At compost maturity, TP increased by 14.9%, 27.4%, and 35.2% in MC_1, MC_2, and MC_3, respectively. This increase was due to the concentration effect resulting from organic matter mineralization and mass loss during composting. As decomposition progresses, carbon is lost as CO_2 while the moisture content decreases, thereby concentrating phosphorus in the remaining substrate (Wei et al., 2017). Additionally, microbial activity promotes the transformation of phosphorus from organic forms into more stable inorganic forms, enhancing its bioavailability to plants. This enrichment is agronomically significant because phosphorus plays a major role in energy transfer, cell division, and the synthesis of nucleic acids and membrane phospholipids (Sánchez et al., 2017). Microorganisms assimilate dissociative phosphorus for their growth and are attracted to low molecular compounds such as proteins and sugars during decomposition. As microbially mediated activity increases, phosphorus mineralization is also enhanced (Wei et al., 2015). The TP content of the compost showed moderate concentration, indicating that the compost was suitable for soil application without contributing to phosphorus overload, as excess phosphorus, especially that in leachable forms, can lead to nutrient leaching and environmental degradation (Hurley et al., 2017; Liu et al., 2014).

The results for total potassium (TK) show that all treatments initially had the same value (4.2 mg/g) but increased to 5.9 mg/g in the final phase of composting (Figure 9c and d). This increase positively correlated with the moisture levels, such that the higher the moisture content, resulted in greater potassium release. Specifically, TK content increased by 1.0 mg/g (19.2%) in MC_1, 0.9 mg/g (17.3%) in MC_2 (55%), and 2.1 mg/g (33.3%) in MC_3 (65%), with average final TK concentrations of 5.0 mg/g, 5.2 mg/g, and 5.9 mg/g, respectively (Figure 9c and d). This consistent trend across the treatments can be linked to the same amount of organic residue used. However, the differences observed later in the process were attributable to moisture-mediated mineralization dynamics. The highest (65%) and moderate (55%) moisture levels may have promoted microbial activity and decomposition of organic matter to facilitate the release of potassium from plant tissues and mineral sources. In addition, the increase in electrical conductivity (EC), although still below the phytotoxic threshold of 4 mS/cm (Figure 4c), might have played an important role. This incidence may have occurred as a result of the accumulation of soluble mineral salts released during organic matter decomposition. However, excessively high EC can impose osmotic stress that may inhibit microbial activity (Bernal et al., 2009).

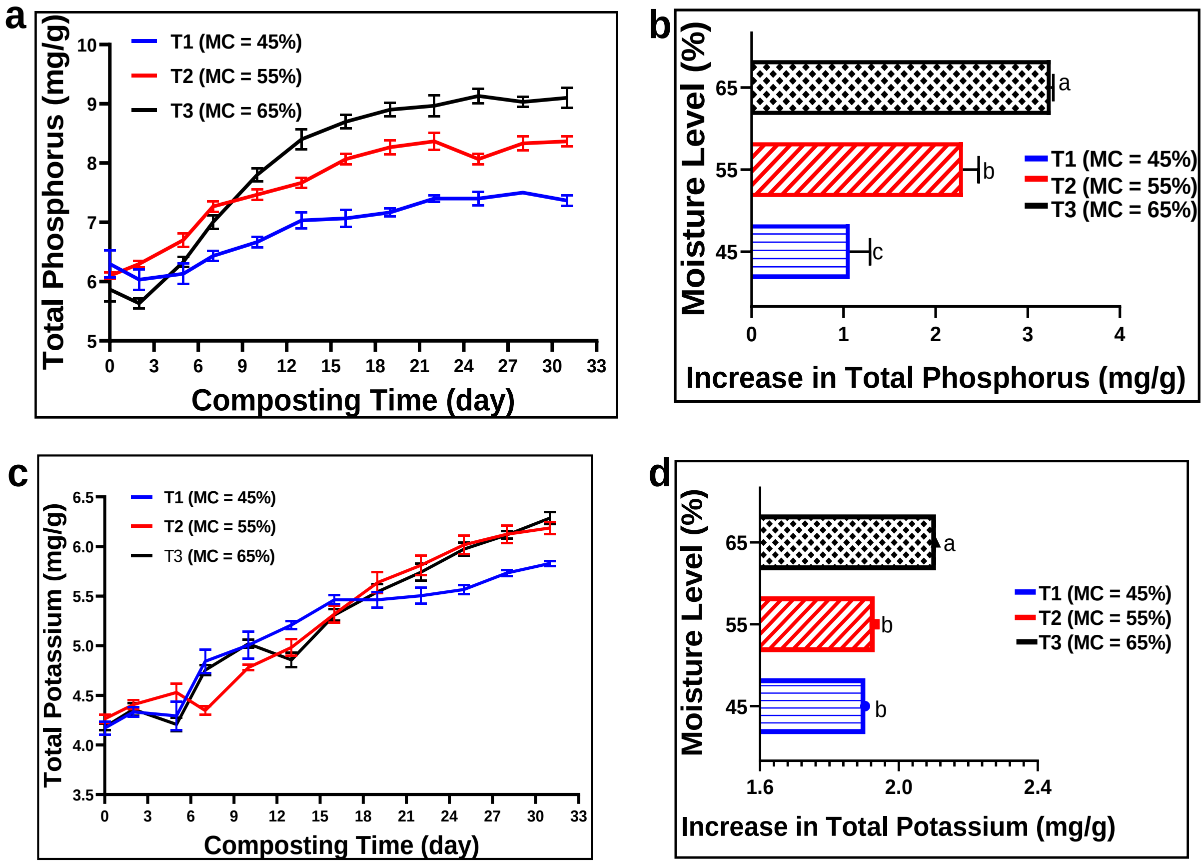

Figure 9. Temporal dynamics of total phosphorus (a and b) and total potassium (c and d) as affected by initial moisture optimization during composting. Error bars indicate ± standard error of means (n = 3)

Variations in C/N Ratio, Germination Index and Moisture Content

As depicted in Figure 10a, a slight increase in carbon to nitrogen (C/N) ratio was observed in all treatments at the beginning of composting, followed by a sharp decline on the third day. The initial increase could stem from the loss of nitrogen, particularly through ammonia volatilization, which was higher than the rate of organic matter decomposition in the early composting stage. This phenomenon temporarily increased the C/N ratio in all treatments. After a sharp increase during the first 3 days, the C/N ratio gradually decreased over time and stabilized at 16.9 for MC_1, 16.4 for MC_2, and 16.1 for MC_3. Compost is typically classified as mature when its C/N ratio falls below 20 (Cui et al., 2017; Wu et al., 2019; Afonso et al., 2021). The steady reduction in C/N ratio reflects the progressive decomposition and mineralization of organic materials with microbial demand for carbon exceeding that of nitrogen, especially within the thermophilic and cooling phases. As a result, the carbon-rich compounds decomposed more rapidly than the nitrogenous compounds.

Figure 10b illustrates the changes in the germination index (GI) of the compost heap across treatments. Statistically, a marked difference was observed between the treatments and the composting duration ($p < 0.03$), showing that both moisture and duration had a strong effect on compost maturity. All treatments showed a gradual increase in GI over time despite the low values obtained in the early phase (mesophilic) of composting. The increasing trend in GI reflects the ongoing degradation and stabilization of organic materials, which improves the compost's suitability for plant growth. The low values recorded could commonly be attributed to high levels of ammonia (NH_3) and volatile organic acids that are typically produced in the preliminary phase of composting (Mao et al., 2019; Selim et al., 2012). These substances inhibit seed germination and root elongation, thus resulting in low GI values. Their gradual decomposition and volatilization over time reduce phytotoxicity, thereby allowing the GI to increase as composting progresses. Upon maturation of the compost, GI showed 70%, 89%, and 96% increases compared to the initial composting period for 45% MC, 55% MC and 65% MC, respectively. This increase emphasizes the crucial role of water adequacy in improving compost maturation and minimizing phytotoxicity. Throughout the composting period, germination index was below 80% for the 45% MC (65%) and 55% MC (78%), but exceeded 80% for the 65% MC (84%). Increases pertaining to the MC_3 could be due to the long temperature phase showed by the treatment. Such occurrence may have enhanced the decomposition of toxic compounds, particularly organic acids and ammonium nitrogen, and reduced pathogenicity. In contrast, the MC_1 had a shorter thermophilic phase which could have affected the decomposition stability and compost maturation (Bernal et al., 2009).

As shown in Figure 10c, moisture content varied considerably ($p < 0.01$) among treatments, with MC_3 exhibiting a modest rise on day 6. The differences are due to the varying moisture contents at the start of composting. This variation was expected as moisture affects microbial respiration and evaporation during composting. In addition, MC_3 with the highest initial moisture level, may have favored internal water redistribution or condensation in the pile, leading to the observed transient increase. Water loss at the early phase of composting was 44% in MC_1, 30.4% in MC_2, and 23.4% in MC_3. These values indicate that a higher initial moisture content helps reduce early water loss and provides suitable conditions for microbial efficiency. The 65% moisture treatment retained more water due to its higher initial content, supporting sustained biological activity and more consistent decomposition. This performance can be attributed to sufficient moisture supporting microbial growth without causing anaerobic condition.

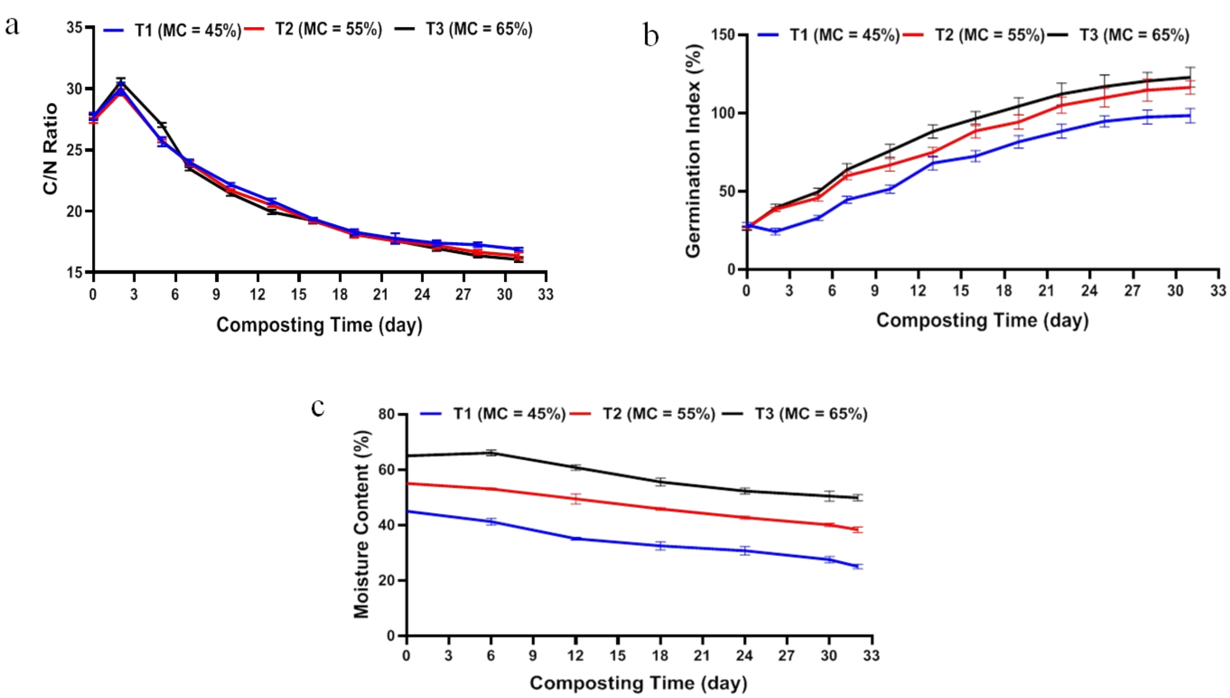

Figure 10. Changes in the carbon-to-nitrogen ratio (a), germination index (b), and moisture content (c) as affected by initial moisture optimization during composting. Error bars indicate ± standard error of means (n = 3)

Mechanism of Water on Substrate Decomposition

Substrate decomposition is largely driven by water (Ghanney et al., 2021). The decomposition begins with the solubilization of organic compounds (Awasthi et al., 2015), where water infiltrates the compost matrix and dissolves readily degradable compounds. This aqueous environment promotes the bioavailability of nutrients and also makes them accessible to microorganisms (Ayilara et al., 2020). As a dissolving and transport medium, water facilitates the diffusion of dissolved nutrients from the compost mass to microbial cells supporting metabolic activities (Figure 11). Maintaining cellular hydration enables optimal enzymatic and biochemical reactions, which in turn promote microbial growth and colonization (Bohacz, 2018). As microbial biomass increases, microorganisms begin to secrete extracellular enzymes such as cellulases, ligninases, and proteases that break down more complex, structurally rigid organic materials (Ghanney et al., 2021). This marks the beginning of the depolymerization phase, in which the polymers are converted into simpler compounds. With continued decomposition, the system transitions to nutrient mineralization (Sharma et al., 2024; Lin et al., 2025), in which organically bound elements like nitrogen, phosphorus, and sulfur are transformed into inorganic forms (ammonium, nitrate, and phosphate). The process then enters the humification phase, where microbial metabolites and partially decomposed residues condense and polymerize to form stable humic substances that ultimately lead to the production of humus (Wu et al., 2017; Guo et al., 2019). Finally, compost reaches maturity when microbial activity declines, odor stabilizes, labile organic matter decreases and the temperature returns to ambient.

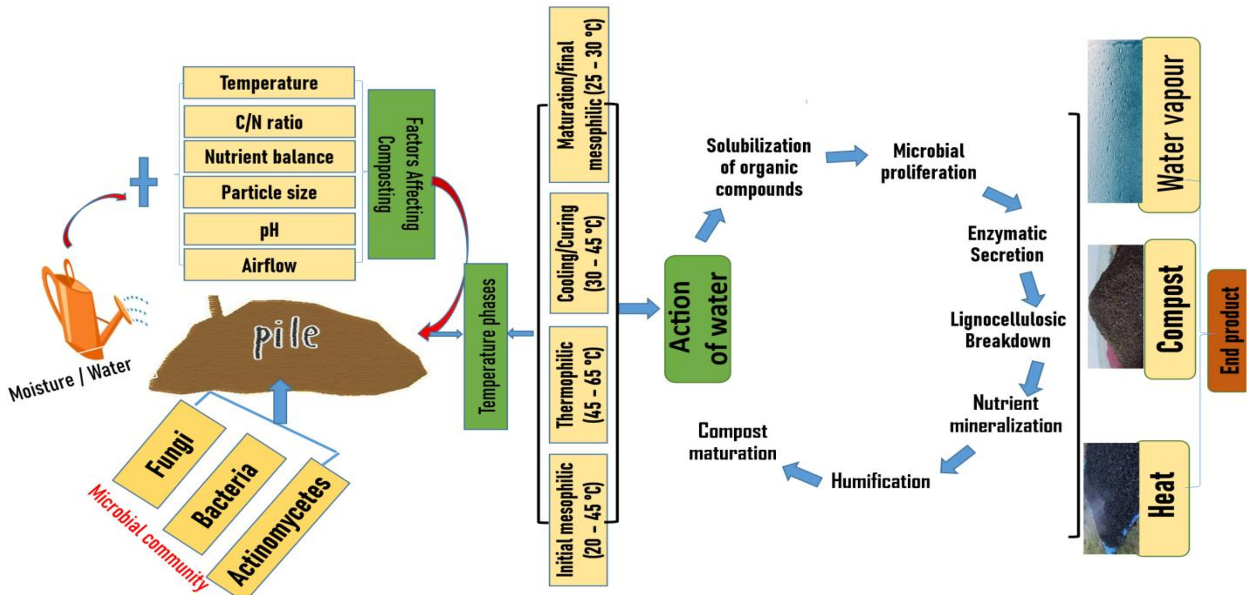

Figure 11. The schematic diagram of the mechanism of water on substrate decomposition

CONCLUSION

This study demonstrates the critical role of initial moisture content in regulating the microbial activity, nutrient dynamics, and overall compost quality during the aerobic composting of corn straw and cow manure. Among the tested treatments (45%, 55%, and 65% moisture content), the 65% moisture level consistently produced superior outcomes, including the greater microbial biomass accumulation, increased nutrient availability, and improved compost maturity. The 65% moisture content promoted more favorable composting conditions, as shown by earlier thermophilic activity and higher values of microbial biomass carbon and nitrogen, dissolved organic nitrogen, and total phosphorus. In addition, the moisture loss exhibited an inverse relationship with the initial moisture content, with the lowest losses observed at 65%. The higher germination index under this treatment further indicated reduced phytotoxicity and a greater degree of compost stabilization. The findings suggest that optimizing the initial moisture content to 65% provides a cost-effective and environmentally friendly alternative to chemical or thermal pre-treatments often used to accelerate composting.

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D.K.A., S.Y., P.A.Y., A.B., M.A.B.; Supervision: X.X.; Project Administration: P.G., H.Q.; All authors have read and agreed to the published version of the manuscript.

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