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ORIGINAL PAPER

Impact of Hydrated Lime Co-additives on Nitrogen Conservation during Livestock Waste Composting

Donggyu Bang[1] · Woojin Chung[2] · JinJu Yun[3] · Jeahong Shim[3] · Byong-Hun Jeon[4] · Mohd Huzairi Mohd Zainudin[5] · Mohammed Junaid Hussain Dowlath[6] · Balasubramani Ravindran[2] · Natchimuthu Karmegam[7] · Soon Woong Chang[2]

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Abstract

Nitrogen loss during the composting process is a great challenge that can lead to environmental pollution and reduce compost quality. Lime is often added to the composting mixture to increase the pH, speed-up the decomposition process, and lower the release of toxic gases like ammonia. However, the specific effects of lime on nitrogen dynamics, particularly ammoniacal nitrogen and nitrate nitrogen levels, as well as CO_2 emissions, remain areas of active investigation. This study investigates the influence of hydrated lime on nitrogen conservation when added to poultry manure and agricultural waste. To evaluate the level of nitrogen retention and overall compost stability, poultry waste and agricultural waste were co-composted with and without hydrated lime amendment under controlled environmental conditions. The results showed that, in comparison to the control, the lime-treated compost had higher nitrate nitrogen levels (1800 mg/kg) and lower ammoniacal nitrogen levels (100 mg/kg), indicating improved nitrogen retention. Furthermore, CO_2 emissions in the compost treated with hydrated lime were higher in the early phases, however substantially dropped as the compost matured, indicating a faster stabilization process. The findings of 16 S rRNA sequencing showed that lime-treated composting was dominated by *Thermobifida*, *Thermobacillus*, and *Saccharomonospora*, all of which were known as cellulolytic bacteria and involved in organic matter degradation. Also, significant bacterial shifts were observed during the thermophilic phase. The *Pseudomonas* population, which is often associated with the denitrification process, was lower than the control, thus, promoting nitrogen retention. The results imply that lime amendment improves composting stability and quality by increasing nitrogen content while reducing organic matter. This work advances the understanding and knowledge on the influence of lime in composting by providing useful insights into the microbial community that can be used for improving the process.

Keywords Composting · Chicken manure · Hydrated lime · Livestock waste · Nitrogen conservation

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Donggyu Bang and Woojin Chung contributed equally to this work.

✉ Balasubramani Ravindran
kalamravi@gmail.com

✉ Soon Woong Chang
swchang@kyonggi.ac.kr

[1] Department of Environmental Energy Engineering, Graduate School of Kyonggi University, Suwon 16227, Korea

[2] Department of Civil & Energy System Engineering, Kyonggi University, Suwon 16227, Korea

[3] Division of Soil and Fertilizer, National Institute of Agricultural Sciences, Rural Development Administration, Wanju 55365, Korea

[4] Department of Earth Resources and Environmental Engineering, Hanyang University, 222- Wangsimni-ro, Seongdong-gu, Seoul 04763, Republic of Korea

[5] Laboratory of Sustainable Animal Production and Biodiversity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Selangor Serdang 43400 UPM, Malaysia

[6] Department of Anatomy, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India

[7] PG and Research Department of Botany, Government Arts College (Autonomous), Salem, Tamil Nadu 636 007, India

Introduction

The population of the world has grown dramatically, reaching 7.9 billion in 2021. It is expected to reach roughly 10 billion by 2050 and 11 billion by 2100 [1]. As a result, there has been an increase in the global need for food, and by 2050, it is expected that the amount of food needed per person would double [2]. According to estimates, 40% of all meat consumed is produced from poultry, with China being the second largest producer with 20 million Mg in 2020. The increased demand for chicken meet has led to a problem of manure treatment [3]. A chicken is estimated to create 80–100 g of droppings every day, or roughly 3–4% of its body weight [4]. The management of livestock waste, in particular chicken manure, is a serious environmental issue due to the rapid expansion of poultry industries *vis-à-vis* production of manure in large quantities resulting in management issues apart from associated pollution in air, water and soil [5–8]. Like other animal excreta, chicken manure is an organic waste rich in nutrients about 3–5% nitrogen, 0.9–3.5% phosphorus, and 1.5-3% potassium [9] and is frequently used as untreated organic fertilizer in croplands. Furthermore, compared to cattle or pig dung, the concentration of calcium, magnesium, and sulphur is significantly higher [10]. However, the improper utilization of poultry manure as fertilizer has associated environmental concerns, such as including air pollution, rising greenhouse gas (GHG) emissions, buildup of dangerous trace metals, and eutrophication in water bodies, acidification of the soil, and increased loss of nutrients, especially nitrogen and phosphorus from the soil through leaching, erosion, and runoff [11].

Apart from livestock crop production has increased significantly to meet the high demands of several million, which has further increased the amount of agricultural waste produced [12]. Over the past century, China, India, and Africa have not only increased the population and economy but also the agricultural residues [13]. Agricultural residues contribute to a significant portion of organic waste generated globally. These residues if not managed properly, can pose environmental risks, including soil degradation and air pollution. Conventional methods to manage agricultural residues and livestock waste include landfilling, incineration and direct application on lands. While landfilling organic waste can lead to the emission of GHGs such as ammonia, carbon dioxide, and nitrous oxide which are harmful to both people and the environment [14]. Direct application on land, though beneficial for soil fertility, often results in nutrient leaching and runoff contributing to water pollution. Whereas burning can effectively reduce the amount of waste and destroy pathogens, it also generates significant air pollution, including particulate matter and toxic gases thereby raising public health concerns [15].

One of the biggest biological sectors with the highest biomass production is agriculture, which can provide a significant bioeconomy input. Bioeconomic strategies based on the management of agricultural residues can stop the underuse of livestock manure and the careless or random burning of crop residues to guarantee food and health security, waste valorization to produce products with added value, farmer's livelihood, youth employment opportunities, and agriculture sustainability [16]. Composting has emerged as a sustainable alternative for managing organic waste, converting it into stable, nutrient-rich compost that can enhance soil fertility, porosity, aeration, and water retention. Therefore, turning agricultural wastes and byproducts into valuable resourceswill not only open up green markets and create jobs, but it will also lessen the need for fossil fuels and GHG pollution, helping to promote clean, safe, and sustainable agriculture [17, 18]. During the composting process, N compounds such as protein are converted into ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), and dinitrogen (N_2) through nitrification and denitrification under aerobic and anaerobic conditions. However, it is important to note that, N loss may happen during the composting process as a result of NH_3 volatilization, leaching of (NO_2^-) and (NO_3^-), and N_2O, and N_2 emissions [13]. To overcome these challenges, several additives have been explored to reduce nitrogen losses and enhance the composting process as well as improve the quality of the compost [19–21]. For instance, 0.5–1.5% lime and 10% calcium magnesium phosphate fertilizer addition on maturity, gaseous emissions, and bacterial dynamics during food waste revealed the highest alleviation in CH_4 (80.77%) and NH_3 (39.85%) emissions during composting [22]. Hydrated lime is one such additive that has been investigated for its potential to mitigate ammonia emissions during the composting process [23, 24]. Previous studies on the use of lime have indicated that hydrated lime increases the pH of the composting material, which can inhibit the urease activity and reduce ammonia volatilization thereby effectively reducing ammonia emissions and improving nitrogen retention [25, 26]. Based on the current literature search, there is limited information available regarding the impact of adding lime on the microbial community during the composting process. Microorganisms drive the transformation of organic matter, such as carbon and nitrogen, during composting. These microbes play a crucial role in the conversion of organic matter, including nitrogen changes. Therefore, in this study, the co-composting experiment of poultry manure and agricultural residues was conducted with or without hydrated lime addition to investigate the effects of lime amendment on the physicochemical properties of co-composting as well as its impact on microbial diversity. This was done to gain a better understanding of microbial properties and theirrole in improving the performance of the composting process.

Materials and Methods

Materials

The chicken manure was obtained from Uhwa farm in Eumseong, Chuncheongbuk-do. The crop residues were obtained from the National Academy of Agricultural Sciences.

Preparation of Composting Substrate and Experimental Design

The experiment was conducted in a room at a temperature of 25 ± 2℃ in Kyonggi University, Suwon, Gyeonggi-do, South Korea. The experiment had had 2 treatment groups labeled as control and lime treatment. To adjust the initial moisture contents of all treatments to 60%, Distilled water was added and mixed. Control contained a mixture of chicken manure, crop residues, and saw dust in a ratio of 6:3:1 with a total volume of 7 kg. The lime treatment included all the control mixtures and 210 g (3%) of hydrated lime. with hydrated lime derived 210 g (3%). All the raw materials were mixed thoroughly and periodically to attain even distribution. All the experiments were conducted in triplicates in-vessel. The composition of the components of each processing unit is shown in Table 1.

Physicochemical and Gaseous Emission Analysis

For the nitrogen conservation and physicochemical evaluation of compost, some common parameters such as temperature, pH, electric conductivity, moisture content, organic matter, and generated CO_2 and NH_3 concentration were determined. The temperature of the compost was measured every day at the same time using a probe-type thermometer through the upper hole of the reactor. The moisture content and organic matter were determined by the change in weight after drying at 105 °C. The pH and electric conductivity were determined by diluting the sample at a ratio of 1:10 (wt%) with distilled water stirred for 30 min and measured using a pH meter (HANNA Co, HI-2222, USA) and an electric conductivity meter (OHAUS Co, STARTER 300 C, USA). The odor gas was measured using a gas detector tube (GASTEC Co, GV-100 S, JAPAN).

DNA Extraction

For gDNA extraction, preserved samples were thawed at 25℃ and thoroughly mixed using a vortex mixer immediately before aliquoting 400 µL each. Aliquots were centrifuged at 10,000 rpm, and the supernatants were discarded. The pellets from each sample were used to extract gDNA using DNeasy PowerSoil Pro Kits (QIAGEN, Hilden, Germany). The bead homogenization process was carried out using a Bead Ruptor Elite (OMNI International, Kennesaw, GA, USA) homogenizer for 1 min at 6 m/s, followed by 1 min rest in a metallic thermal bead bath from 80℃ storage for cooling the heat from the homogenization in between the homogenization steps for a total of 3 min of homogenization. The gDNAs were quality-checked using a Nanodrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and stored at 80℃ until further application.

16S rRNA Amplicon Library Preparation and Sequencing

The gDNA extracts were diluted to 2.5 ng/µL, and 2 µL of each dilution was used for PCR amplification. PCR amplification of the V3–V4 hypervariable region of the 16 S rRNA gene was performed using 10 pmol in 1 µL each of 341 F forward primer (5′– A-Adaptor– Barcode–Stuffer–CCTACGGGNGGCWGCAG– 3′) containing IonCode barcode sequences provided by Ion Torrent protocol and 805 R reverse primer (5′– P1-Adaptor– GACTACHVGGGTATCTAATCC– 3′) [19], both procured from the oligonucleotides synthesis service (Macrogen, Seoul, South Korea). Platinum™ PCR SuperMix High Fidelity (Thermo Fisher Scientific, Waltham, MA, USA) was used for the PCR master mix. The PCR reaction conditions were as follows: initial enzyme activation of 180 s at 94 ◦C (Stage 1), followed by 25 cycles each of 30 s denaturation at 94 ◦C, 30 s annealing at 50 ◦C, and 30 s extension at 72 °C (Stage 2), and final extension at 72 °C for 5 min (Stage 3). The produced V3–V4 amplicons were purified using HiAccuBead SPRI bead (Accugene, Incheon, South Korea) with the library to magnetic bead ratio of 1:1.5 in a magnetic rack and washed twice with 70% ethanol before elution with 30 µL of 0.1 M Tris-EDTA buffer. The purified amplicon concentration was

Table 1 Feedstock composition ratio of treatment compost

Treatment	Feedstock ratio (% wet weight)					Additive (% raw feedstock on wet weight)
	Crop residues (%)			Chicken manure (%)	Saw dust (%)	Hydrated lime (%)
	Sesame stalks	Pepper stalks	Bean stalks			
Control	10	10	10	60	10	-
Lime	10	10	7	60	10	3

Table 2 Physicochemical characteristics of raw materials used for composting

Parameters	Crop residues			Chicken manure	Saw dust
	Sesame stalks	Pepper stalks	Bean stalks		
Moisture contents (%)	7.95 ± 0.48	8.01 ± 0.21	7.54 ± 0.32	51.40 ± 1.32	8.30 ± 0.25
Organic matter (%)	96.69 ± 1.52	95.65 ± 1.38	91.95 ± 1.47	76.95 ± 2.21	98.69 ± 0.98
Ash (%)	4.01 ± 0.09	8.53 ± 0.16	7.44 ± 0.17	11.20 ± 0.12	1.20 ± 0.15
T-C (%)	48.67 ± 1.32	50.31 ± 2.34	50.65 ± 1.57	44.11 ± 1.48	53.24 ± 1.68
T-N (%)	1.78 ± 0.12	2.21 ± 0.15	1.26 ± 0.09	3.05 ± 0.24	ND
T-P_2O_5 (%)	0.21 ± 0.08	0.31 ± 0.05	0.19 ± 0.02	3.15 ± 0.15	0.01 ± 0.005
T-K_2O (%)	0.27 ± 0.09	0.31 ± 0.04	0.19 ± 0.03	2.14 ± 0.18	0.03 ± 0.003
pH	7.36 ± 0.15	6.66 ± 0.14	6.03 ± 0.07	7.55 ± 0.15	6.78 ± 0.17

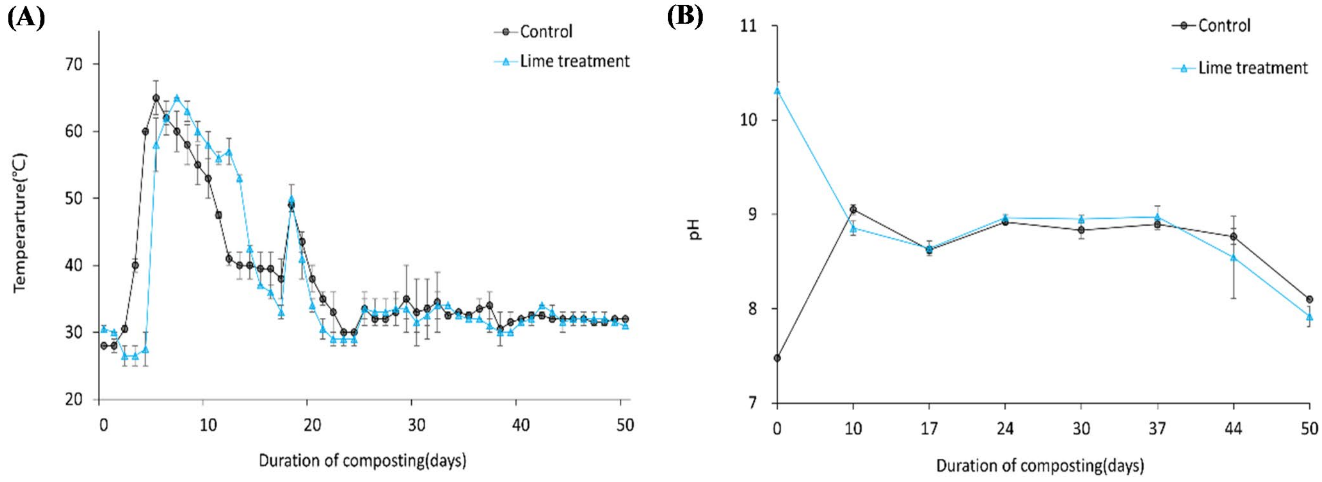


Fig. 1 Dynamics of (**A**) temperature and (**B**) pH in the lime-treated composting group

checked using Invitrogen Qubit 4 fluorometer dsDNA assay kit (Thermo Fisher Scientific, Waltham, MA, USA), and the samples were pooled in equimolar concentration to a total concentration of 100 pmols in 50 µL of the final library. The prepared final library was then used for template preparation in Ion Chef Instrument (Thermo Fisher Scientific, Waltham, MA, USA) using Ion 520™ and Ion 530™ ExT Kit– Chef (Thermo Fisher Scientific, Waltham, MA, USA) and Ion 530™ semiconductor chip (Thermo Fisher Scientific, Waltham, MA, USA) with the procedures described in the Quick Reference document (Pub. No. MAN0015806 Rev. E.0). Near the end of template preparation, the Ion GeneStudio S5 System (Thermo Fisher Scientific, Waltham, MA, USA) was cleaned and initialized using ExT sequencing reagents following the manufacturer's protocol, and the prepared Ion 530 chip was loaded for the sequencing run. After sequencing, the demultiplexed FASTQ files were retrieved from the Torrent Suite™ server for bioinformatic processing.

Statistical Analysis

General data visualization and plotting were conducted using Sigma Plot 14.0. Bacterial community analysis was carried out using R version 3.1.21 and subsequently visualized through Sigma Plot. These tools were applied via the Galaxy platform (www.freebioinfo.org). Phylogenetic diversity analysis and co-occurrence networks of key genera were constructed using the online tool MicrobiomeAnalyst 2.0.

Results and Discussion

Physico-chemical Properties of Raw Materials

Table 2 provides a summary of the physical and chemical properties of the raw materials that were analyzed using various parameters.

Temperature Dynamics

Figure 1(A) shows the dynamics in the temperature profile of control and lime-amended composting, indicating the effects of lime amendment on the pattern of degradation. Both treatments experienced rapid temperature increases during the initial phase. There was a peak thermophilic phase followed by a cooling phase in the temperature dynamics.

As a result of vigorous microbial activity and efficient decomposition of organic matter, the high temperature was maintained for several days. From the 13th day onwards, temperature gradually decreased and reached around 32 °C and 31 °C for control and lime amendments, respectively, indicating the maturation and stabilization of compost. The addition of lime influenced the temperature profile, resulting early and prolonged thermophilic phase compared to the control. This could be attributed to the exothermic reaction of lime when it hydrates, and releases excess heat during the composting process [27]. It is essential that both treatments maintain high temperatures during the thermophilic phase in order to reduce pathogens and break down complex organic compounds [28]. As the composting process progresses, the temperature gradually declines, indicating the transition to the maturation phase, when microbial activity slows and the compost stabilizes [29]. However, as the composting progressed a change in temperature profile was observed.

Effect of Lime Treatment on the Physicochemical Characteristics during Composting

pH Dynamics

The pH of compost is a critical parameter influencing microbial activity, nutrient availability and overall quality of the compost. In this study, the pH of both control and lime-amended mixtures was monitored throughout the composting process to assess the impact of hydrated lime amendment. The pH of the control mixtures exhibited a typical composting pH profile where the initial pH was 7.48. By the 10th day, the pH increased sharply to 9.05, which may be due to the microbial decomposition of organic matter and the production of ammonia (Fig. 1B). The amendment of commercial lime to the mixture significantly influenced the pH profile. The initial pH of the test treatment was 10.31 which is due to the alkaline nature of lime. The biotransformation of nitrogenous compounds, such as proteins, uric acids, and amino acids resulted in a significant generation of NH_4^+, which was subsequently transformed into NH_3 to neutralize the acids produced within the composting piles. This led to the resultant pH observed in both treatments [30]. The pH of the control mixtures then gradually decreased to 8.1 by the 50th day, showing stabilization as the composting process progressed. By the 10th day, the pH of lime amendment decreased to 8.85, indicating a buffering effect. The pH fluctuated slightly reaching 7.92 by the 50th day, stabilizing earlier than the control treatment. The pH dynamics observed in both treatments align with previous reports that have examined the effects of lime on composting processes [31]. Given that lime is an alkaline substance, it is remarkable that pH increased more noticeably in the lime-amended treatment than in the control.

Electrical Conductivity Dynamics

An important parameter for assessing compost's suitability for agricultural use is its electrical conductivity, which measures the amount of soluble salts in compost [32]. In this study, the EC of both control and lime-treated compost mixtures was monitored throughout the composting process to evaluate the impact of hydrated lime amendment. The EC values of both the control and lime-amended mixtures showed a decreasing trend, with EC reaching its lowest point at 4.17 and 4.38 mS/cm by the end of the composting process, indicating some variability in soluble salt content due to lime amendment (Fig. 2A). The initial sharp decrease in EC observed in both treatments can be attributed to the leaching of soluble salts and the consumption of these salts by microbes during the decomposition of organic matter [33]. The degree of salinity in the compost and any potential phytotoxicity effects on plant growth are reflected in the EC value of the compost. Higher EC in the finished compost will hinder seed germination and limit the flow of nutrients and water into the plants [34]. However, in the present study reduction in EC was observed similar to that of the trend of EC in lime-amended compost [35]. Furthermore, lime amendment is known to promote organic humification thereby facilitating the polymerization of salts, organic acids, and low molecules for EC reduction [36].

Moisture Content

The moisture content of both treatments was monitored to assess the impact of lime on moisture retention and management. Figure 2(B) shows the moisture content during the composting process. The moisture content in the control and lime treatments, began at 55.03 and 56.15%, respectively, on the 0th day. As the composting progressed, an increasing trend was observed in both treatments. By the 10th day, the moisture content had increased to about 59% and 58% in control and lime treatments, respectively. The moisture content was maintained between 55 and 65% throughout the composting process with the control treatment showing higher moisture on days 10 to 30 compared to the lime-amended mixture. The optimum moisture content for optimum microbial activity for degradation of organic matter during the composting process is around 60–70% [37]. At the end of the composting period, the control's moisture content was 64%, while the lime-treated mixture retained a high moisture level of 63% compared to the control.

This higher moisture retention observed in lime-treated mixtures can be because of the hygroscopic nature of lime,

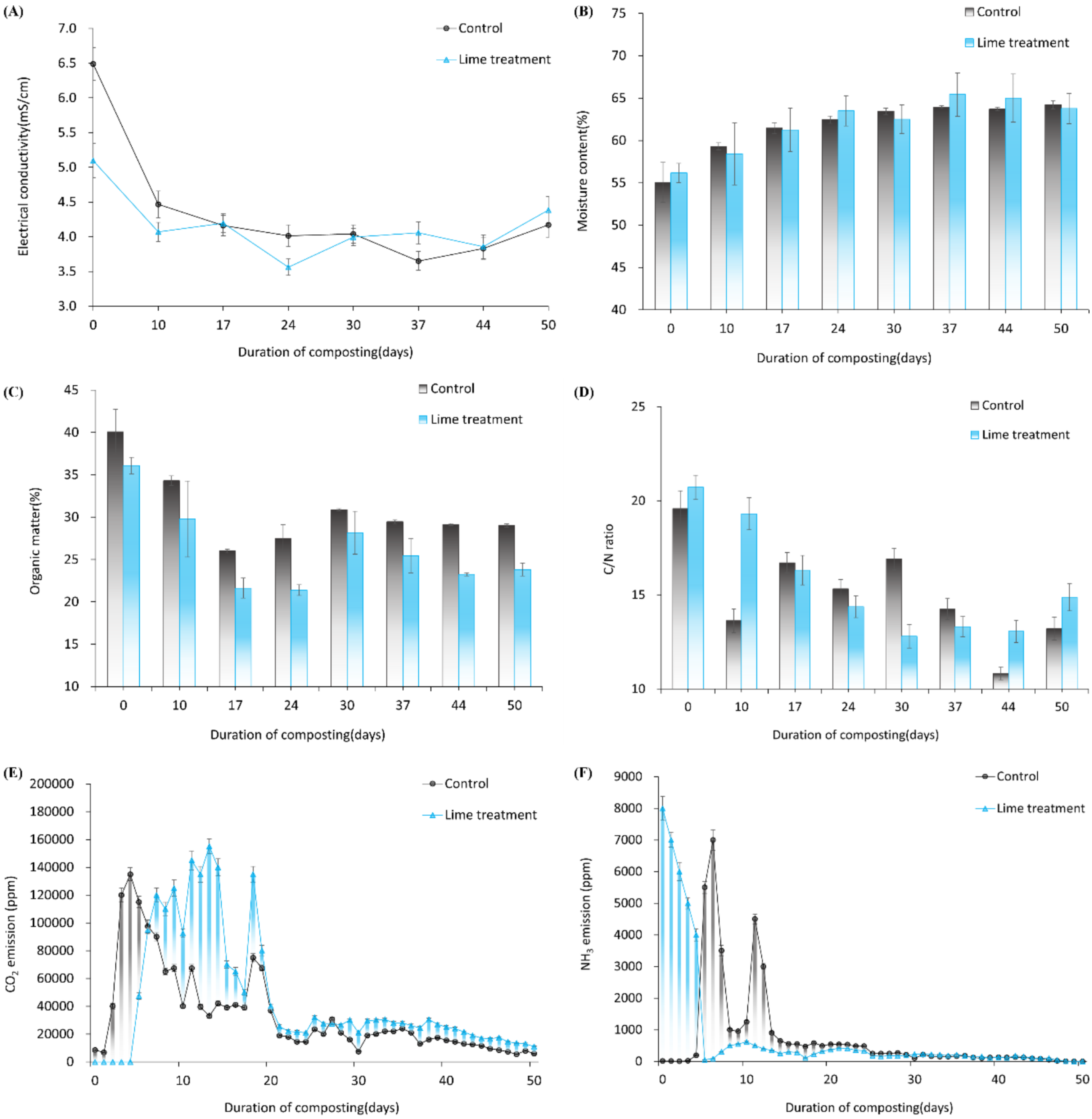


Fig. 2 Profile of physicochemical parameters: (**A**) electrical conductivity, (**B**) moisture content, (**C**) organic matter, (**D**) C/N ratio, (**E**) carbon dioxide emission, and (**F**) ammonia emission during the composting process (bars denoted as ±standard deviation)

which helps to retain the moisture in the compost. Moisture retention is crucial to maintain microbial activity and for the effective breakdown of organic matter [38]. The correlation between moisture content and temperature is evident in this study. Higher temperatures can cause more evaporation and therefore a decrease in moisture content was observed. Despite the higher temperatures experienced with the lime treatment, there was a higher level of moisture retention, possibly because lime is hygroscopic. Another possible reason could be the maintenance of pH level, where ammonium ions become unstable and are released as ammonia gas [34]. This balance between moisture retention, temperature, and pH indicates a more controlled and efficient composting process in the lime-amended mixture.

Organic Matter Content

The initial organic matter content of bothtreatment mixtures was recorded. The organic matter content in both treatments significantly declined as composting progressed (Fig. 2C). Microbial activity during composting is responsible for this reduction observed in both mixtures. On day 10, the lime-amended mixture experienced a more substantial reduction of about 20%, compared to 13% for the control. It was noted that the reduction trend continued throughout the composting period, with the control mixture showing a more gradual decrease than the lime-amended mixture. By the end of composting, the organic matter in the control mixture decreased to 29% whereas the lime-amended mixture had a lower content of 24%. This higher rate of organic matter reduction in lime-treated mixtures is due to the enhanced microbial degradation facilitated by the alkaline conditions of lime [39]. The higher temperatures also facilitated the breakdown of complex organic compounds into simpler forms, thereby reducing the overall organic matter content [40]. Furthermore, the maximum reduction in organic matter observed in lime-treated mixtures was associated with the higher mineralization of organic matter than that of control. In general, organic matter content decreased and the mature compost normally contains organic matter below 30% [41], which aligned with the results observed in our study.

C/N Ratio

The rate at which micoorganisms decomposes organic matter is significantly influenced by the C/N ratio in compost. The initial C/N ratio for both control and lime-amended compost were 19 and, 22 respectively (Fig. 2D). If the initial C/N ratio is high, the initial decomposition process will be slowed leading to consumption of longer duration for maturation [42]. However, the lime-amended compost experienced a reduction in the C/N ratio by the end of the composting process. In this study, the addition of lime to chicken manure and agricultural waste resulted in a higher C/N ratio when compared to the control treatment without lime. The supplement of a nitrogen source such as chicken manure and agricultural waste in the presence of lime could have accelerated the decomposition process. In general, the rate of decomposition with high initial C/N ratio was faster than the compost with a low initial C/N ratio. Compost with a C/N ratio below 20 is considered to be proper [43, 44], however, ranges between 10 and 15 indicate a good degree of maturity of the compost [45]. The C/N ratio of the lime-treated compost obtained in this study aligns with the C/N ratio value of 15.

Gaseous Emissions

CO2 Emissions

The rate of CO_2 emissions can be used to quantify the microbial activity in the compost [46]. High oxygen demand and CO_2emission ratesindicate the instability of the compost [47]. Since the stability of the compost affects nitrogen immobilization and phytotoxicity, it is a crucial component to evaluate the quality of compost. The CO_2 emission rates began relatively low for both treatments, with 8500 and 0 ppm for control and lime-treated mixtures, respectively (Fig. 2E). In the initial composting, CO_2 emission observed in the lime-amended mixture is an indication of delayed microbial activity, which is expected since the lime creates an alkaline environment that temporarily inhibits the microbial activity until acclimatization [48]. As the composting progressed, the control mixture experienced its peak of 115,000 ppm on the 5th day. Whereas, lime-treated mixtures exhibited a slower initial increase in CO_2 emissions, peaking on the 11th day at 145,000 ppm, surpassing the control. The CO_2 emissions in the lime-treated mixture continued to greater peaks reaching 155,000 ppm on day 13, while control remained significantly low at 33,000 ppm. The delays of CO_2 generation are typical characteristics because lime is being used. Lime initially inhibits the microbial activity but then stimulates it more effectively as the environment stabilizes with changes in pH that support the decomposition of organic matter at an accelerated rate [49]. Then the emissions in both mixtures steadily decreased and reached 550 and 1000 ppm for control and lime-treated mixtures, respectively. This indicates that the compost has reached stability.

NH3 Emissions

In addition to CO_2 emissions, NH_3 emissions are a significant event and the main source of nitrogen loss that lowers the quality of the compost [50]. NH_3 is a notable indirect GHG that causes malodors and contributes to environmental pollution and global warming. It was suggested by Onwosi and colleagues that lime amendments in composting mixtures could mitigate global warming [26]. A high level of NH_3 emission was observed in both treatments during the thermophilic phase (Fig. 2F). A pH increase is known to increase NH_3 emissions, particularly between 6.0 and 8.8. A pH of around 7.5 enhances the NH_4^+/NH_3 ratio, which increases the release of NH_3 [51]. The organic nitrogen compounds in the composting mixture are rapidly mineralized to NH_4^+, which is released as NH_3 during the thermophilic and alkaline phases of composting.

In this study, the control treatment showed high NH_3 emissions, peaking at 7000 ppm on Day 6. This is because of an increase in pH which causes intensified decomposition and accumulation of NH$_4^+$ leading to significant emissions of NH$_3$. The lime-treated mixtures initially exhibited high NH$_3$ emissions which then rapidly declined falling to just 50 ppm by day 5. This decline is advantageous and can be attributed to the rapid formation of stable compounds such as struvite and the interaction of NH$_3$/NH$_4^+$ with lime additives, which reduces NH$_3$ volatilization [31]. This is facilitated by the alkaline condition created by lime amendment and it aligns with the pH trend observed in our study. Over the composting period, the lime-amended mixture exhibited lower NH$_3$ emissions compared to the control, thus, promoting N retention. The lime-treated mixtures reduced overall NH$_3$ emissions by a significant margin, highlighting its effectiveness in mitigating nitrogen loss during composting, making the final product higher in nutrients. Further, this is beneficial in minimizing the environmental impact caused by NH$_3$ emissions [52].

Nitrogen Conservation

Ammoniacal Nitrogen

The NH$_4^+$-N and NO$_3^-$-N contents during the composting were also noted. The initial levels of NH$_4^+$-N were found to be very high in the control mixtures compared to lime-treated mixtures. Over the composting period, both the treatments experienced a decline which was more pronounced in the lime-amendedmixture. The findings showed that at the early stages of composting, in lime-amended mixtures, the quantities of extractable NH$_4^+$-N increased before rapidly stabilizing at the end of composting. The rapid decline of the lime-amended mixture as shown in Fig. 3(A) could be due to the enhanced microbial activity and the buffering effect of lime [53], which helps in regularizing the pH and promoting the conversion of NH$_4^+$ to NO$_3^-$ instead of volatilization as NH$_3$ [26]. These results show that lime amendment reduces nitrogen losses through volatilization and contributes to the overall nutrient stability of the compost. The results of the present study are consistent with previous reports on lime amendment in composting food and kitchen waste [54, 55]. Further, the lower ammoniacal nitrogen levels observed in lime-treated mixtures highlight its importance in the effective management of nitrogen forms, making it a suitable material to reduce nitrogen loss and enhance the quality of the final product.

Nitrate Nitrogen

The dynamic differences in the NO$_3$—N content between both treatments are given in Fig. 3(B). At the initial stage, the control mixture had low nitrogen levels (22 mg/kg), which increased very gradually and reached 770 mg/kg by the end of the composting period. Whereas, the lime-treated mixture at the initial stages showed 190 mg/kg which increased and reached 1790 mg/kg by the end of composting. This significant difference is due to the influence of pH and microbial activity. The increased pH in lime-treated mixtures observed during the initial stages enhances nitrification which is more efficient in higher pH. This pH favors the nitrifying bacteria that convert ammonium into nitrate ($NH_4^+ \rightarrow NO_3^-$) [56]. It also implies that limeamendment not only accelerates the nitrification process but also helps in overall nitrogen conversion [23]. The peak nitrate level of 179 mg/kg implies that lime effectively accelerated the nitrogen conversion processes, leading to increased stability and maturity of compost when compared to control. Higher nitrate concentrations are often associated with more mature compost, which is beneficial for agricultural applications due to its nutrient availability and stability [57].

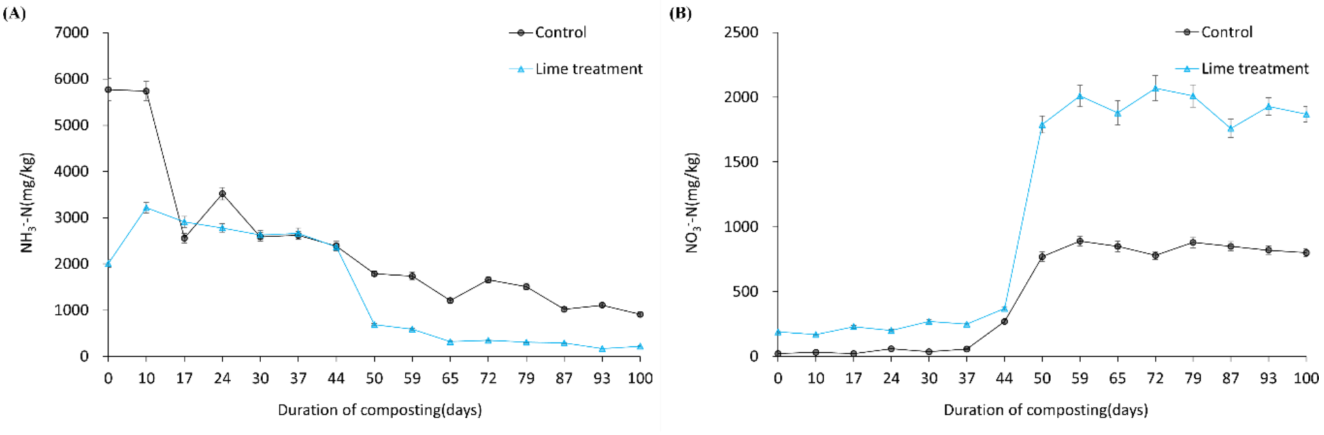


Fig. 3 Profile of NH$_3$-N (**A**), and NO$_3$-N (**B**) in the lime-treated composting group

Comparison of the Taxonomic Compositions

Phylum Level

Taxonomic analysis of the samples at the phylum level showed that Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidota were dominant (Fig. 4A). When lime was applied to compost, the relative abundance of Proteobacteria decreased compared to the control (22.50% and 15.58%, respectively) where Proteobacteria are known for their significant roles in organic matter decomposition and Actinobacteriota increased (23.29 to 31.85%, respectively) which also plays a key role in breaking down complex organic materials. During the composting period, Firmicutes which help in nitrogen recycling commonly increased in the thermophilic phase (47.88–67.21%), and compared to the control group, the lime treatment group showed a higher abundance (51.22 to 93.43%). The dominance of Firmicutes and Actinobacteriota during the mesophilic phase is consistent with their role in the degradation of complex organic matter during the early stages of composting, particularly under lower temperatures [58]. Lime treatment appeared to reduce the presence of certain phyla like Bacteroidota, while increasing the abundance of Planctomycetota and Patescibacteria, which may indicate a shift in microbial activity related to the alkaline conditions introduced by lime. Compared to the control group, Firmicutes and Gemmatimonadota accounted for a higher proportion in the lime treatment group. This can be confirmed by clearly distinguishing between the lime treatment group and the control group due to the change in temperature profile according to lime injection in terms of the compost community structure. In the curing phase, the control group showed a revival of Actinobacteriota and Proteobacteria, indicating a stabilization of microbial communities as the temperature decreased. The lime-treated compost displayed a similar trend, with Actinobacteriota and Proteobacteria maintaining their presence. Chloroflexi and Bdellovibrionota were also notable in the lime treatment, which suggests diversification of microbial populations due to improved nitrogen retention and the less acidic environment [59]. During the maturation phase, the lime-treated group displayed a higher proportion of Actinobacteriota, suggesting better organic matter degradation and nitrogen conservation. The lime treatment may also promote the growth of microorganisms involved in humification, contributing to the overall quality of the compost [52]. These results indicate that the application of lime leads to distinct differences between the lime-treated and control groups, attributed to changes in temperature profiles and microbial community structures. It potentially favours the microbes that survive in more alkaline environments and shifts the balance of those involved in nutrient cycling, particularly nitrogen and carbon decomposition. This microbial shift could be correlated with the observed differences in nitrogen retention and emissions since some microbes are more efficient in ammonia oxidation or nitrification under certain pH levels.

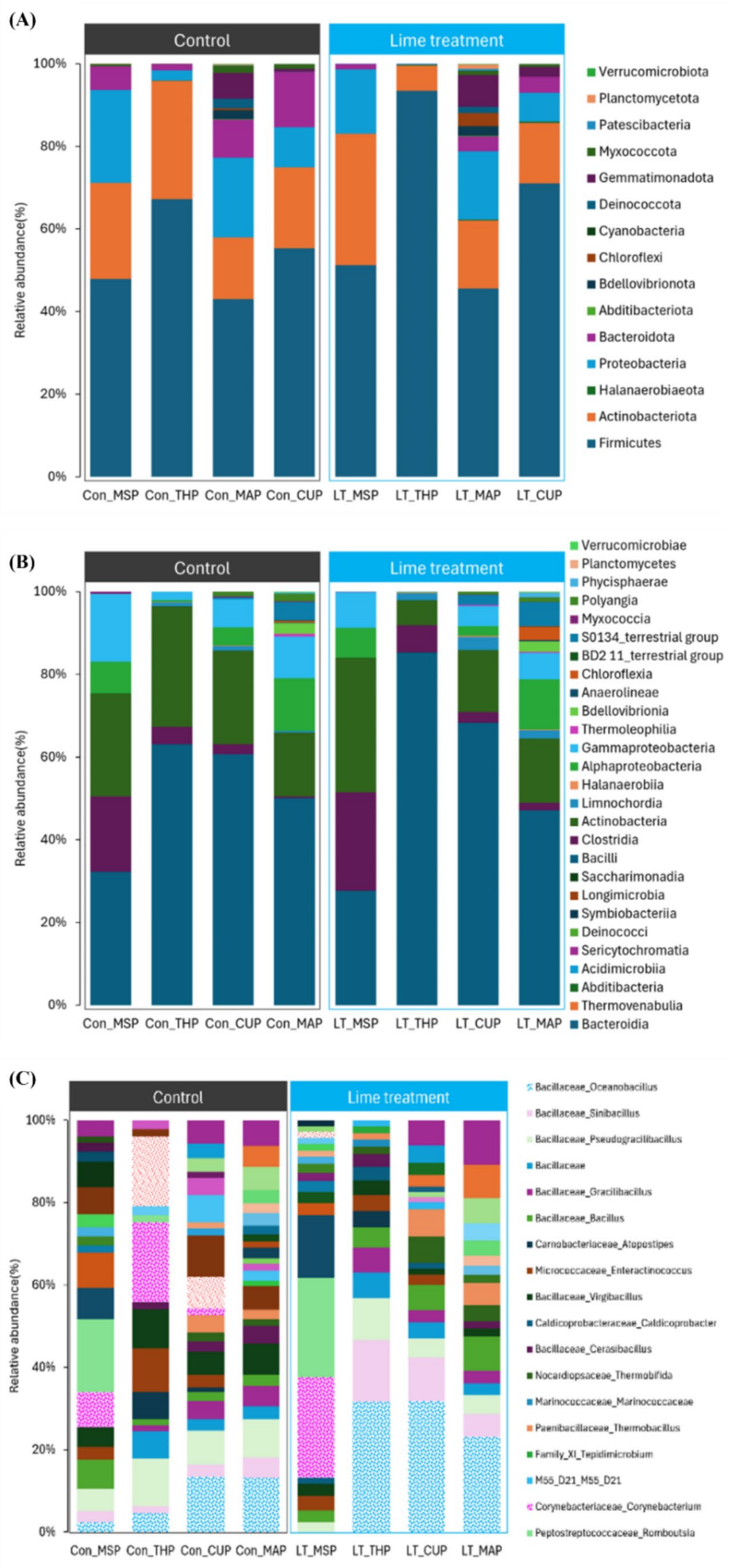
Class Level

The taxonomic analysis at the class level shows the dynamics in microbial community structure in both treatment. Heat tree analysis was performed for a better understanding of the community structure (Fig. 5A & B). During the mesophilic phase, in the control treatment, Bacilli (30.21) and Actinobacteria (23.28) were the most abundant classes followed by Clostridia (17.09) and Gammaproteobacteria (15.32) (Fig. 4B). Anaerolinae and Thermoleophilia were also present in smaller proportions which indicates the diversity even in oxygen-limited environments. In the lime-amended treatment, similar dominant classes with significant shifts were observed during the mesophilic phase. Actinobacteria (31.84) and Clostridia (23.28) increased while Bacilli (27.09) and Gammaproteobacteria (8.53) decreased which is likely due to the rapid pH changes induced by lime, which create an alkaline environment affecting microbial activity and nutrient utilization.

During the thermophilic phase of the control group, an increase in actinobacteria (28.67) and bacilli (62.06) was observed which gradually decreased in the maturation phase (13.08 and 42.32). Actinobacteria plays a key role in the decomposition of complex organic matter such as cellulose and lignin, particularly in the early and midstages of composting. Whereas in the limeamended group, Actinobacteria, Clostridia and Bacteroidia decreased (6.11, 6.59, and 1.55, respectively) while Bacilli increased to 85.22 and stabilized to around 42.25 by the end of the maturation phase. The lime amendment altered the conditions for anaerobic microbes, potentially by enhancing aeration or increasing pH. The sharp increase in Bacilli during the thermophilic phase, especially in the lime-treated group, indicates that this class is highly adapted to high temperatures and alkaline conditions, playing a crucial role in the breakdown of organic matter during composting [60].

In the control group, Actinobacteria and Clostridia decreased and nearly disappeared by the curing and maturation phases. The decline of Clostridia suggests that this class is more active in the initial breakdown of organic matter under anaerobic conditions but cannot tolerate extreme heat or alkaline conditions during the thermophilic phase. Whereas Bacilli remained high in the curing and maturation phases. Gammaproteobacteria and Bacteroidia that dropped during the thermophilic phase began to recover during curing and continued to increase during the maturation phase.

Fig. 4 Taxonomic analysis of thecontrol andlime-treated composting group: (**A**) Phylum-level, (**B**) Class-level, and (**C**) Genus-level



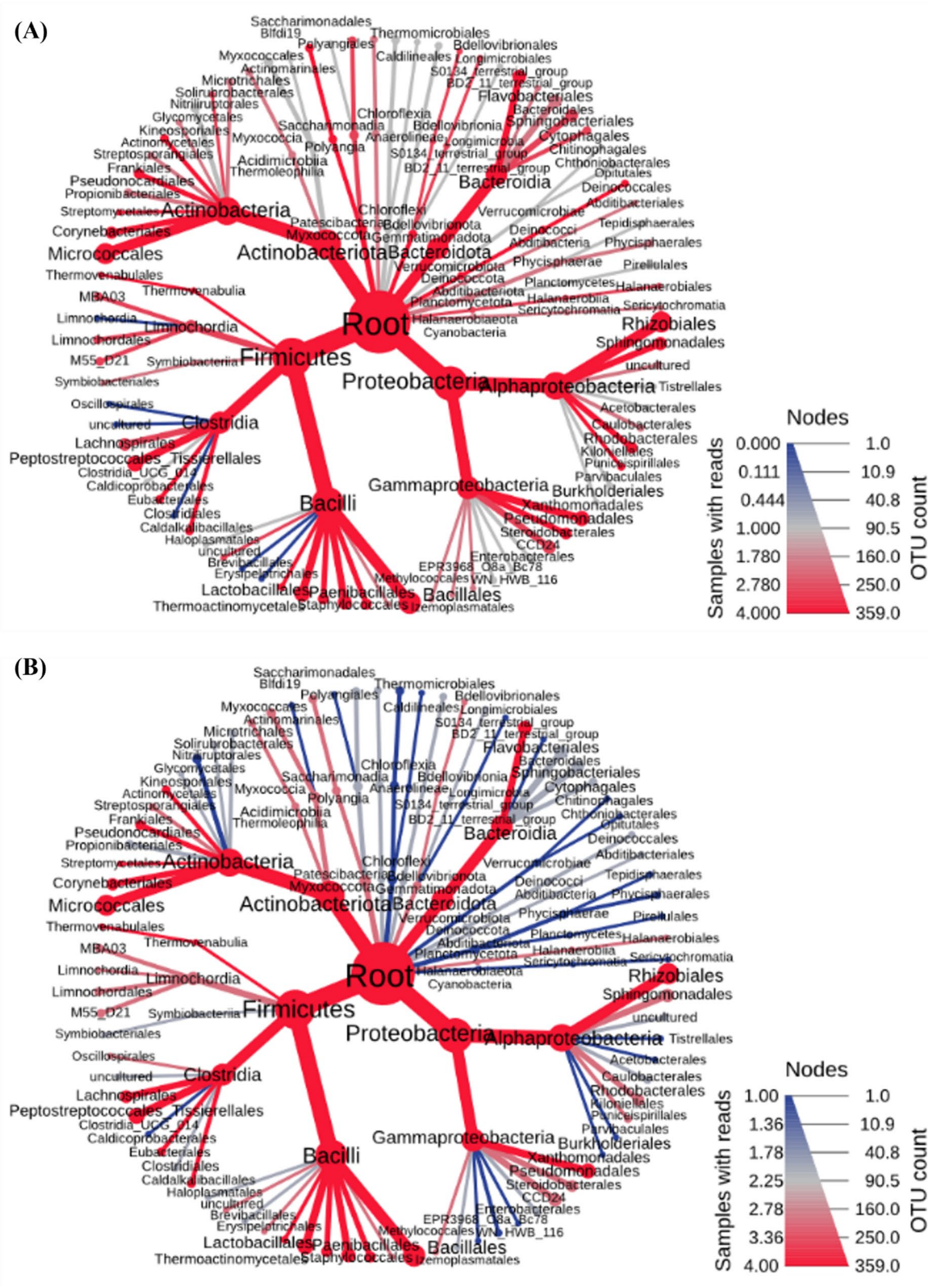


Fig. 5 Heat-tree analysis of (**A**) Control and (**B**) Lime-treated composting group

In the case of lime-amended treatment, Bacilli followed similar trends as in the control group but Actinobacteria, Alphaproteobacteria and Gammaproteobacteria slightly recovered during the maturation phase (14.06, 10.86 and 5.74). This trend suggests that Actinobacteria are sensitive to both high temperatures and alkaline conditions, especially during the thermophilic phase. However, they tend to recover in the later stages when conditions become more stable and favorable for their growth. The recovery of Alphaproteobacteria indicates itsrole in nitrogen stabilization during the cooling down and stabilization phases of composting. The decrease in Bacteroidia is beneficial as it helps suppress pathogenic microorganisms, reduce unpleasant odors, and improve compost quality. Microbes that survive in neutral to slightly acidic conditions including Bactroidia, struggle to survive in the high pH environment caused by lime amendment. The alkaline conditions disrupt the peptidoglycan layer and outer membrane structure of microbes, damaging their cell walls and impairing their functions [61]. The alkaline environment further favors the growth of beneficial microorganisms like Firmicutes and Actinobacteria, which compete with Bacteroidia for nutrients, enhancing organic matter decomposition efficiency. The reduction in Bacteroidia also mitigates the spread of antibiotic resistance genes [62], resulting in safer compost for agricultural use.

Genus Level

The genus-level taxonomic analysis of the samples highlighted the high diversity of microbiota in both treatments (Fig. 4C). A notable feature was the significant genus-level distinction between the control and lime-amended treatments. During the mesophilic phase, in the control group, genera such as *Peptostreptococcaceae_Romboutsia* (13.04) and *Corynebacteriaceae_Corynebacterium* (6.26) were highly abundant. *Romboutsia* and *Corynebacterium* are typically involved in early-stage organic matter breakdown, particularly proteins. The lime-treated mesophilic phase showed similar diversity with elevated relative abundance of both the genera *Romboutsia* (17.19) and *Corynebacterium* (17.47), reflecting its versatility across varying pH levels and its role in early composting processes.

The thermophilic phase, characterized by elevated temperatures, exhibited significant bacterial shifts. In the control group, *Corynebacterium* (17.41%) and *Romboutsia* (1.62%) remained prominent. However, these genera were absent in lime-treated samples suggesting its sensitivity to alkaline conditions caused by lime addition. Conversely, in the lime-treated group, alkaliphilic genera such as *Oceanobacillus* (29.50%) and *Sinibacillus* (13.89%) were dominant, highlighting their resilience and adaptive advantage in alkaline conditions. Additionally, *Pseudogracilibacillus* (9.47%), suggesting that lime addition fosters a thermophilic bacterial community better suited for enhanced decomposition and nitrogen retention. It is particularly known for its ability to degrade complex organic substrates under high-temperature, alkaline conditions [63], further promoting efficient composting during this phase. Moreover, *Atopostipes* (5.92%) were only found in control while *Thermobifida* (1.65%) and *Caldicoprobacter* (3.00%) appeared exclusively in lime-treated groups. This indicates that lime promotes thermophilic, cellulolytic, and proteolytic bacteria are capable of accelerating organic matter degradation and nitrogen conservation under the higher temperature and pH conditions of the thermophilic phase. As the compost enters the maturation phase, microbial activity slows, and the community shifts to bacteria that help in stabilizing the compost. In the control genera such as *Oceanobacillus* (11.53%), Flavobacteriaceae (8.66%) and *Sinibacillus* (2.51%) maintained a significant presence, suggesting their involvement in the final stages of organic matter decomposition. In contrast, the lime-treated group displayed a microbial community enriched with thermophilic and alkaliphilic genera. *Oceanobacillus* (14.99) remained highly abundant along with *Thermobifida* (2.49) and *Thermobacillus* (3.53). The persistence of these genera throughout the thermophilic and maturation phases reflects their role in maintaining nitrogen conservation and stabilizing the compost. Additionally, the presence of *Saccharomonospora* (6.99%) in lime-treated samples suggests that these actinomycetes are favored in alkaline environments and may contribute to the final breakdown of recalcitrant compounds. The genera *Pseudomonas* and *Corynebacterium*, while abundant in the early and thermophilic phases of the control treatments, were largely absent in the lime-treated samples, indicating their sensitivity to alkaline conditions created by the lime addition. The decline of these genera in the lime treatments could contribute to a more efficient nitrogen conservation process, as *Pseudomonas* species are often associated with denitrification [64], which leads to nitrogen loss in compost. The overall effect of lime treatment promotes a microbial community that favors nitrogen conservation and organic matter breakdown, particularly in alkaline and thermophilic conditions, aligning with the composting goals of enhancing nitrogen retention and reducing environmental emissions.

Alpha Diversity Analysis

Figure 6 represents the comparative analysis of the alpha diversity of control and lime amended composts. This provides the details about the richness and evenness of the microbiota in the two different treatments. In this, the Chao1 index indicates the species richness. During the early stages of composting, Chao1 index of both control and lime

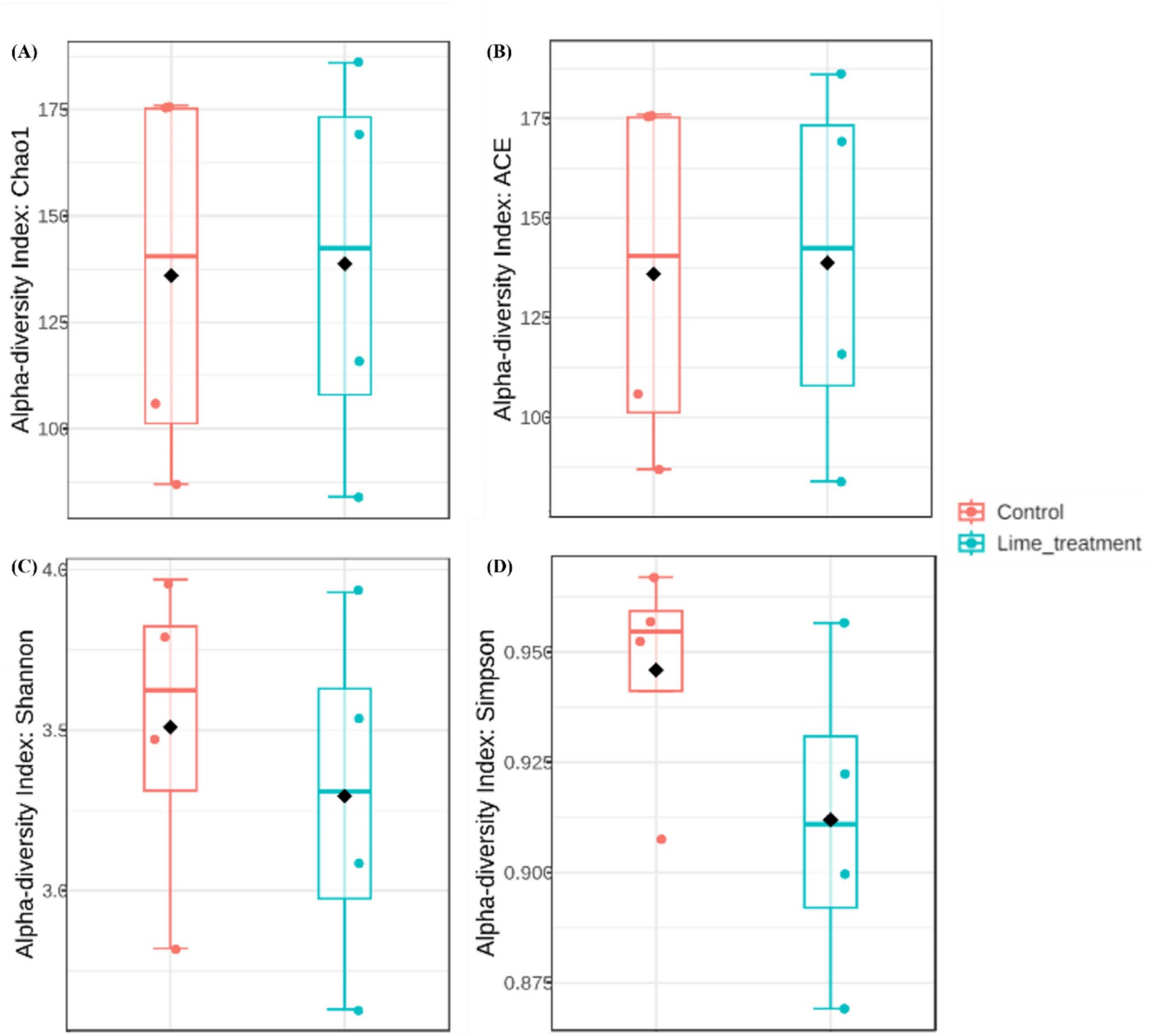


Fig. 6 Alpha diversity analysis of control and lime-treated composting group - (**A**) Chao1, (**B**) ACE, (**C**) Shannon and (**D**) Simpson

treatments had a high and comparable species richness. By day 10, there was a notable decline in species richness in both the treatments, but the lime-amended treatment exhibited slightly lower Chao1 index compared to the control. By day 17, species richness in both treatments increased again, where lim-amended treatment showed slightly higher Chao1 index. The lime-treated group potentially supported the growth of new, alkaliphilic species, which could explain the slightly higher species richness at this point. By the end of composting, species richness returned to or exceeded their initial values in both the treatments, again with slightly higher in lime-treated compost. This could indicate that the lime treatment allowed for the survival and growth of a more diverse set of microbes adapted to higher pH levels by the end of the composting process [29]. Similar to Chao1, the ACE index also estimates the species richness. The distribution of ACE values are found to be similar to that of Chao1. Since the values of the lime-amended treatment is wider, it indicates more viability in richness. This also reflect the changes in microbiota due to lime amendment that supports certain organisms that has the ability to survive in higher pH levels.

Shannon index combines both richness and evenness [65]. At the beginning of the composting process, Shannon index showed slightly higher diversity in the control than the lime-amended treatment. By day 10, both the treatments experienced a notable decline in Shannon diversity, with lime amendment showing slightly lower than the control.

This recovered by day 17, with control treatment showing Shannon index slightly higher than the lime-amended treatment. The lower Shannon index in lime-amendment suggests a pronounced uneven community, in which a few dominant species might thrive while others are less represented. Whereas, by the end of composting period, Shannon diversity returns to levels close to or even below the initial values in both the treatments, with slightly higher in lime-amendment, which suggests that, over time, both treatments foster a diverse microbial ecosystem, though the trajectory differs.

The Simpson index focuses more on evenness and the dominance of species. The Simpson index measures the probability that two individuals randomly selected from a sample will belong to the same species, with values closer to 1 indicating higher diversity. During the initial stages, the Simpson index was higher in control group than lime-amended group indicating an evenly distributed microbial population in the control group. Further, both the groups experienced a similar decline which was recovered in the later stages of the control group but remained low in lime-amended group. By the end of composting period, Simpson index of both the groups were similar to that of initial stages with slightly higher values in control than lime-amendment. However, the difference between both the groups is very minimal. Overall, the control group maintains the microbial population more uniformly dispersed during the composting process, but the lime treatment seems to have a higher selective influence on microbial diversity, especially in the early and mid-phases. The microbial community in the lime-treated compost is more diverse, with certain species favoring the alkaline conditions, resulting in a decrease in overall diversity. This implies adding lime amendment affects the microbial environment by encouraging alkaliphilic species to predominate while suppressing others, especially those that are susceptible to higher pH values.

Conclusions

As a co-additive to livestock waste composting, hydrated lime has been shown to enhance nitrogen conservation. In composting, poultry manure supplies vital nutrients like nitrogen, phosphorus, and potassium that promote microbial activity. Maintaining the ratio of nutrients was possible with agricultural waste, which served as a useful carbon source. Lime amendment in co-composting poultry manure and agricultural waste provided an ideal environment for microbial activity, speeding up the decomposition process and ensuring nutrient-rich compost. The reduction of ammonia emissions led to a lower ammoniacal nitrogen level and a higher nitrate level in the final product. The C/N ratio of 15 in lime-treated compost indicates the maturity of the end product. The co-composting process improves compost quality while minimizing pollution to the environment. Overall, the addition of hydrated lime offers a potential approach to maximize the poultry waste composting procedures, supports environmental friendly waste management techniques, and produces high-quality compost.

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Data Availability All data used for the research are provided in the article.

Declarations

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

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