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*Full Length Article*

# Microbial Dynamics and Nutrient Transformation in Liquid Organic Fertilizer Produced from Organic and Manure Waste

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## Abstract

The increasing global demand for sustainable agricultural practices has driven the development of eco-friendly alternatives to chemical fertilizers. This study investigated the microbial dynamics and nutrient transformations during the production of Liquid Organic Fertilizer (LOF) using organic waste and manure. The objective was to evaluate shifts in microbial populations and nutrient profiles, specifically organic carbon, nitrogen, phosphorus, and potassium during fermentation. The fermentation process was analyzed at three stages: day 0 (POC1), day 12 (POC2) and day 24 (POC3), with metagenomic sequencing performed to examine microbial shifts and nutrient content. Results revealed a significant reduction in organic carbon, accompanied by a decrease in the carbon-to-nitrogen (C/N) ratio, indicating microbial utilization of carbon as an energy source. Nitrogen content progressively increased due to the microbial conversion of ammonia into nitrate. Phosphorus exhibited fluctuating levels, peaking at day 12 and decreasing by day 24, while potassium levels steadily increased**.** Microbial analysis showed a shift from bacterial dominance (Firmicutes and Proteobacteria) in POC1 to fungal dominance (Ascomycota) in POC3, with Penicillium potentially playing a key role in lignocellulose degradation. These microbial shifts were essential for transforming organic matter into a nutrient-rich fertilizer. This study highlighted the importance of microbial succession in optimizing LOF production and its potential to improve the sustainability of agricultural practices. Future research should focus on controlling potential pathogens in LOF for safe agricultural use.

**Keywords:** Fermentation; Liquid organic fertilizer (LOF); Microbial dynamics; Metagenomic analysis; Nutrient transformation

## Introduction

The global human population has grown rapidly, increasing the demand for food. Projections estimate that global food production must increase 70% to meet this demand by 2050 (Dijk *et al*. 2021). This presents challenges to the agricultural sector, which is crucial for food security (Rozaki 2021). Fertilizers, particularly chemical ones like urea, diammonium phosphate, and single superphosphate, are widely used to address nutrient deficiencies in soils (Prakash and Bhargava 2016; Ali *et al*. 2021; Tuncsoy 2021) and boost crop growth (Aryal *et al*. 2021; Penuelas *et al*. 2023). However, prolonged use of chemical fertilizers has led to environmental issues, including pollution of soil, water, and air, as well as the accumulation of toxic heavy metals like cadmium, lead, and chromium, which can enter the food chain (Maqbool *et al*. 2020; Akhter *et al*. 2021; Naz *et al*. 2022).

To address these environmental concerns, the agricultural community has increasingly explored sustainable alternatives, such as organic fertilizers. These fertilizers derived from agricultural and animal waste, release nutrients gradually, improving soil health and biodiversity while reducing reliance on synthetic chemicals (Wei *et al*. 2020; Shaji *et al*. 2021). Meanwhile, organic waste, particularly food waste, contributes to landfill accumulation, methane emissions, and leachate, exacerbating climate change (Biyada *et al*. 2021). In parallel, livestock manure remains underutilized in many developing regions, despite its high nutrient content and potential as a raw material for organic fertilizer production. Utilizing both food and animal waste in fertilizer production offers a promising strategy for integrated waste management and sustainable agriculture.

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Liquid Organic Fertilizer (LOF), produced through the fermentation of organic waste and manure, offers a sustainable alternative to chemical fertilizers. Microbial communities involved in LOF fermentation play a critical role in nutrient transformation by converting complex organic materials into bioavailable nutrients. The fermentation process is influenced by environmental factors such as pH, temperature, and substrate availability (Zhang *et al*. 2023). Nitrogen-fixing bacteria convert ammonia to nitrate, increasing nitrogen content, while phosphorus and potassium are released during fermentation, both essential for plant growth.

Despite the advantages of organic fertilizers, optimizing microbial communities during fermentation remains a challenge. Initially, bacteria dominate by breaking down simple organic compounds, while fungi such as Ascomycota and Basidiomycota take over to degrade more complex polymers like lignin (Huang *et al*. 2015). Understanding microbial roles and their succession is essential for ensuring effective LOF production. Concerns about pathogenic microorganisms, such as Salmonella, Fusarium, Gibberella, remain, as they can pose risks to plant health and human safety if not controlled (Masimbula *et al*. 2019; Gunasekaran *et al*. 2021; Xu *et al*. 2022a).

This study aimed to investigate microbial dynamics and nutrient transformations during LOF fermentation, using metagenomic analysis to track shifts in bacterial and fungal communities at three key stages (days 0, 12 and 24). It also sought to explore potential risks from pathogenic microorganisms, emphasizing the need for safe LOF production. By employing metagenomic sequencing, the research provides insights into microbial interactions during fermentation, addressing a gap in the literature on how microbial processes influence the final product's quality. Understanding microbial succession and nutrient cycling through metagenomic data will help optimize LOF production and control pathogenic microbes, ensuring both safety and efficacy. The findings contribute to sustainable agricultural practices by promoting organic waste recycling, reducing reliance on chemical fertilizers, and addressing microbial risks in LOF production.

## Materials and Methods

### Organic waste and manure collection

The LOF production was sourced from fruit and vegetable waste collected from the Giwangan Market in Yogyakarta, Indonesia. Meanwhile, manure waste from cattle breeders in Mlati, Sleman, Indonesia, was utilized as a key ingredient. The organic waste was carefully selected and processed to ensure a consistent substrate for fermentation. The manure was also processed to remove large debris before use.

### Preparation of LOF

For each LOF reactor, 6 kg of the mixture was prepared, maintaining a 1:1:1 ratio of fruit and vegetable waste, manure, and water. The fruit and vegetable waste was sliced into pieces approximately 0.5 to 1.0 cm in size to facilitate microbial breakdown during fermentation. To enhance microbial growth and accelerate the fermentation process, 20% (w/v) brown sugar was added as an additional carbon source. Brown sugar acts as an external energy source for microorganisms, thereby promoting a faster fermentation rate (Wei *et al*. 2020).

The prepared mixture was placed into fermentation reactors, which were sealed with a lid and connected to a water-filled bottle *via* a hose. This setup created an anaerobic environment conducive to microbial fermentation. The fermentation was allowed to proceed for 24 days, during which microbial activity converted the organic waste into a nutrient-rich fertilizer.

### Fermentation monitoring

The fermentation process was monitored at three key stages: day 0 (POC1), day 12 (POC2) and day 24 (POC3). Samples were taken from the reactors at each stage to analyze the nutrient content and microbial diversity. Environmental parameters, such as pH and temperature, were measured daily to ensure optimal fermentation conditions. The temperature of the fermentation mixture ranged between 25°C and 42°C, while the pH fluctuated between 4.6 and 5.4, ensuring that the microbial community could function efficiently in the desired environment (Febria *et al*. 2023; Giehl *et al*. 2023; Mahongnao *et al*. 2024).

### Microbial community analysis

Metagenomic analysis was used to assess the microbial community present at each stage of fermentation. DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB) method (Creager 2020; Schenk *et al*. 2023). The purity of the extracted DNA was verified through 2% agarose gel electrophoresis. For microbial profiling, the extracted DNA was amplified using 16S rRNA primers for bacteria and ITS2 primers for fungi. Polymerase chain reaction (PCR) amplification was performed, and the PCR products were analyzed using a 2% agarose gel electrophoresis. Sequencing was carried out using a next-generation sequencing platform to acquire high-quality data (Green and Sambrook 2019).

The resulting sequences were processed using FLASH software (version 1.2.7) for assembling paired-end reads (Nihalani and Aluru 2016). Operational taxonomic units (OTUs) were generated by clustering sequences with a 97% similarity threshold, and taxonomic identification was performed using the SILVA database for bacteria and UNITE for fungi (Nilsson *et al*. 2019). Furthermore, the sequence data were analyzed using QIIME2 for microbial community composition analysis (Bolyen *et al*. 2019; Rai *et al*. 2021). Principal Coordinate Analysis (PCoA) was

performed using RStudio to assess microbial diversity and to understand the shifts in microbial populations over time (Wang *et al*. 2022). This helps to highlight the relationship between microbial dynamics and nutrient transformations.

**Nutrient analysis**

The nutrient content of LOF was assessed at each fermentation stage to evaluate its suitability as a plant fertilizer. The organic carbon content was determined using the Walkley-Black method (Morais *et al*. 2019). Total nitrogen was quantified using the Kjeldahl method (FAO 2021), while phosphorus ($P_2O_5$) was measured using the Bray-I method (Pfahler *et al*. 2020). Potassium ($K_2O$) was determined using the 25% HCl flame photometric extraction method (Ullah *et al*. 2022). All nutrient measurements were performed in triplicate to ensure accuracy. The nutrient analysis was conducted at the Agricultural Research and Technology Laboratory in Yogyakarta.

In addition to the nutrient analysis, the pH and temperature of the LOF solution were measured to assess the fermentation environment. These physical parameters influence microbial activity and the breakdown of organic matter. The pH was measured using a pH meter, and the temperature was recorded using a thermometer. These analyses complement microbial analysis and nutrient profile data, offering a complete understanding of the fermentation dynamics.

**Data analysis**

The microbial diversity indices, including the Shannon and Simpson indices, were calculated to assess microbial richness and evenness across the fermentation stages (Feranchuk *et al*. 2018). The Chao1 and ACE indices were also calculated to estimate microbial richness, and the Goods coverage index was used to evaluate sequencing depth and ensure the completeness of the data. The phylogenetic diversity (PD) whole tree metric was used to assess the evolutionary diversity of the microbial communities (Faith 2018).

## Results

**Nutrient analysis of LOF**

The nutrient content of the LOF was assessed at three fermentation stages to monitor changes in organic carbon (C), nitrogen (N), phosphorus ($P_2O_5$) and potassium ($K_2O$). The analysis revealed a significant decrease in organic carbon content. Alongside this, a sharp decrease in the carbon-to-nitrogen (C/N) ratio was observed, which decreased significantly in POC2, then remained relatively stable in POC3. Nitrogen content showed a progressive increase throughout the fermentation period. Following this trend of nutrient transformation, phosphorus content peaked in POC2 but decreased in POC3. In contrast, potassium content steadily increased across the fermentation period. Table 1 summarizes nutrient changes and physical properties, including pH and temperature, during fermentation.

**Metagenomic analysis of microbial communities**

A metagenomic analysis was performed to assess the microbial dynamics across three stages of LOF fermentation. A total of 3.947 bacterial Operational Taxonomic Units (OTUs) and 307 fungal OTUs were identified. The highest number of bacterial OTUs was found in POC1 (1.434), followed by POC2 (1.279) and POC3 (1.234), whereas fungal OTUs were highest in POC3 (137), followed by POC1 (91) and POC2 (79), with an average fungal OTU count of 102 across all samples (Fig. 1a, b). Firmicutes and Proteobacteria were the predominant bacterial phyla in POC1, with Bacteroidota becoming more prominent in POC3 (Fig. 2a). Meanwhile, Ascomycota and Basidiomycota were the dominant fungal phylum throughout the fermentation process (Fig. 2b).

**Venn diagram and microbial diversity**

The Venn diagram (Fig. 3) shows the distribution of bacterial and fungal OTUs across the three fermentation stages. A total of 266 bacterial OTUs were shared across all stages, with POC1 exhibiting the greatest bacterial diversity, while the fungal OTUs showed a more dynamic shift, with POC3 exhibiting the highest fungal OTU diversity. This highlights the evolving role of microbial communities as fermentation progressed. POC1 contained the highest number of unique bacterial OTUs, whereas POC3 had the highest number of unique fungal OTUs.

**Microbial abundance trends**

Bacterial abundance, as shown in Fig. 4a, exhibited a decreasing trend from POC1 to POC3, with POC1 having the highest bacterial abundance, followed by POC2 and POC3. In contrast, fungal abundance increased over the fermentation period, with the highest fungal abundance observed in POC3 (Fig. 4b).

**Heatmap analysis and genus-level composition**

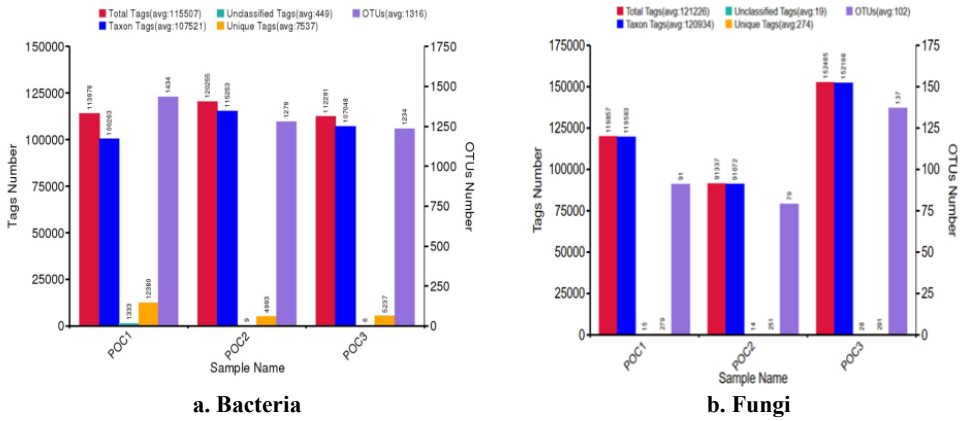
Heatmap analysis (Fig. 5) further clarified the microbial shifts at the genus level during fermentation. In POC1, genera such as Lactobacillus, Pediococcus, and Streptococcus were dominant. In POC3, the dominant genera included Pichia, Hanseniaspora, and Penicillium.

**Microbial diversity indices**

Both Shannon and Simpson indices were calculated for bacterial and fungal diversity across the fermentation stages.

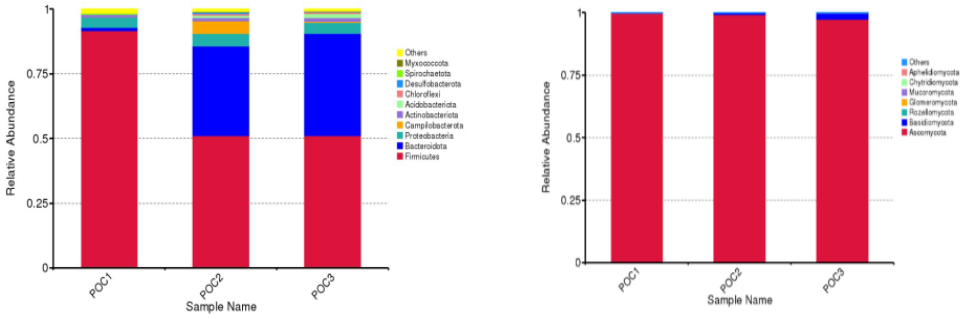
**Table 1:** Analysis of nutrient content and physical properties of LOF samples

| Sample | C-Organic (%) | Total N (%) | $P_2O_5$ (mg. $kg^{-1}$) | $K_2O$ (%) | pH | C/N ratio (%) | Temperature (°C) |
|---|---|---|---|---|---|---|---|
| POC1 | 2.950 | 0.072 | 0.060 | 0.200 | 5.4 | 40.97 | 27 |
| POC2 | 0.970 | 0.093 | 0.400 | 0.220 | 5.1 | 10.43 | 42 |
| POC3 | 1.350 | 0.132 | 0.130 | 0.420 | 4.6 | 10.23 | 26 |



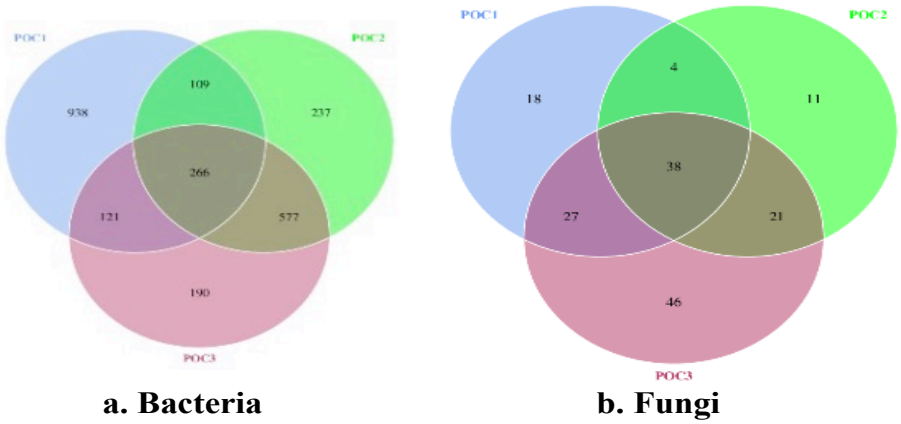

**a. Bacteria** **b. Fungi**

**Fig. 1:** Total bacterial and fungal OTUs in the LOF samples




**a. Bacteria** **b. Fungi**

**Fig. 2:** Bacterial and fungal relative abundance at the phylum level from the LOF samples




**a. Bacteria** **b. Fungi**

**Fig. 3:** Bacterial and fungal Venn diagram of OTU clustering from the LOF samples

Table 2 indicates that the bacterial Shannon and Simpson diversity indices increased over time. However, these values decreased in POC3, particularly in the Chao1 and ACE indices. In contrast, Table 3 reveals a decline in fungal diversity indices in POC2, followed by an increase in POC3, suggesting a rise in fungal diversity.

**Table 2:** Bacterial diversity indices

| Sample name | Observed species | Shannon index | Simpson index | Chao1 index | ACE index | Goods coverage | PD whole tree |
|---|---|---|---|---|---|---|---|
| POC1 | 1434 | 4.210 | 0.785 | 1547.823 | 1593.199 | 0.997 | 248.091 |
| POC2 | 1189 | 5.090 | 0.910 | 1275.506 | 1280.328 | 0.998 | 107.953 |
| POC3 | 1154 | 5.464 | 0.943 | 1209.927 | 1224.691 | 0.999 | 112.965 |

**Table 3:** Fungal diversity indices

| Sample name | Observed species | Shannon index | Simpson index | Chao1 index | ACE index | Goods coverage | PD whole tree |
|---|---|---|---|---|---|---|---|
| POC1 | 87 | 1.239 | 0.422 | 102.400 | 103.640 | 1.000 | 23.805 |
| POC2 | 74 | 1.325 | 0.386 | 79.077 | 80.766 | 1.000 | 24.134 |
| POC3 | 132 | 2.073 | 0.631 | 152.036 | 163.158 | 1.000 | 32.569 |

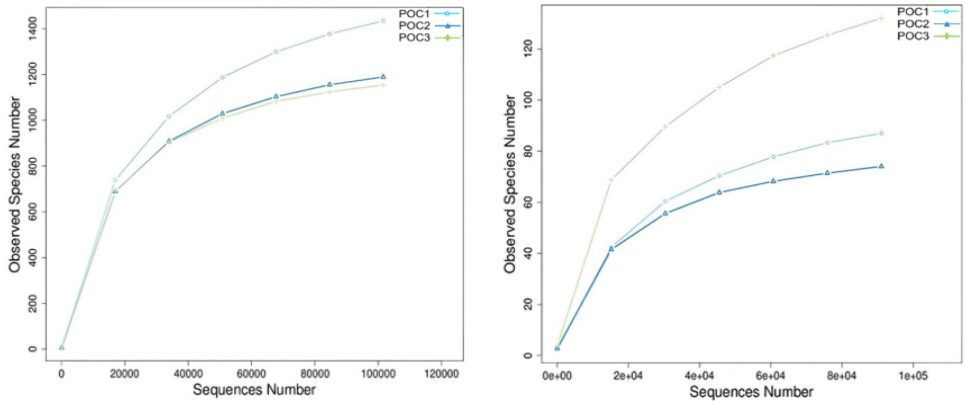



**Fig. 4:** Bacterial and fungal abundance curves from the LOF samples

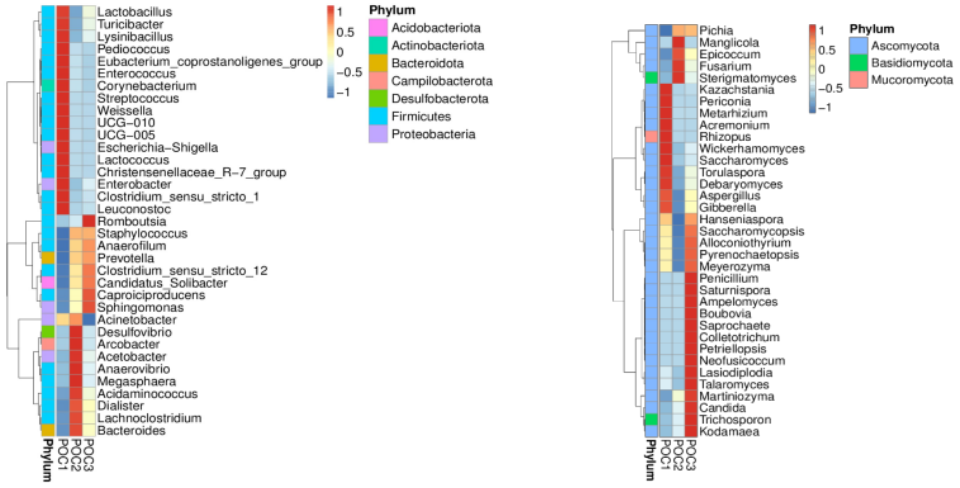



**Fig. 5:** Bacterial and fungal heatmap analysis from the LOF samples

## Discussion

The results showed significant shifts in microbial communities and nutrient profiles, both of which were crucial for optimizing LOF production and sustainability. A decrease in organic carbon and the corresponding reduction in the C/N ratio during fermentation indicated that microbial activity consumed organic carbon for energy, which is essential for compost maturation and nutrient cycling (Peteghem *et al*. 2023; Ma *et al*. 2024). These findings correlated with Purba *et al*. (2024), who demonstrated similar nutrient transformations in liquid organic fertilizer. The sharp decrease in the C/N ratio, followed by stabilization, reflected efficient nitrogen fixation and ammonia conversion to nitrate, supporting nutrient release (Akratos *et al*. 2017). Nitrogen content increased as

nitrifying bacteria facilitated the two-step process of converting ammonia to nitrite and then to nitrate, enhancing nutrient availability (Lesik *et al*. 2019). Phosphorus fluctuated, peaking at day 12 and decreasing by day 24 due to microbial absorption and limited nutrient availability (Yerizam *et al*. 2022). Potassium steadily increased, indicating that microbial activity, particularly the breakdown of potassium-rich organic matter, contributed to its release. According to Yerizam *et al*. (2022), this process is likely due to microbial growth and the mineralization of organic matter. Potassium plays a critical role for plant growth and stress tolerance (Shaji *et al*. 2021).

Metagenomic analysis provided further insights into microbial community dynamics. Bacteria such as Firmicutes and Proteobacteria dominated the early stages, with Bacteroidota emerging in later stages (Zhang *et al*. 2023). Firmicutes are known for their lignocellulose-degrading capabilities, producing hydrolytic enzymes critical for bioconversion (Ransom-Jones *et al*. 2017; Gavande *et al*. 2021). As fermentation progressed, Bacteroidota, which plays a key role in degrading complex carbohydrates, increased, likely due to the transition to cellulose and xylan as primary carbon sources in the later stages of fermentation (Aguilar-Paredes *et al*. 2023). Ascomycota and Basidiomycota fungi became more prominent over time, where ascomycota is well-known for its ability to degrade hemicellulose through enzymes such as endo-1,3-beta-glucanase (Challacombe *et al*. 2019; Wijayawardene *et al*. 2021) and Basidiomycota plays a critical role in lignin degradation (Elisashvili *et al*. 2023). This progressive increase in fungal populations is indicative of the transition from decomposing simpler organic compounds to more recalcitrant materials (*e.g*., lignin), which is essential for nutrient transformation (Zhao *et al*. 2022). The shift from bacterial to fungal dominance aligned with known microbial succession patterns during organic matter decomposition (Hernández-Lara *et al*. 2022).

The Venn diagram revealed that bacterial OTUs showed considerable overlap across all fermentation stages, with POC1 exhibiting the greatest bacterial diversity. As fermentation progressed, fewer bacterial species contributed to the process, and bacterial OTUs decreased over time. In contrast, fungal diversity increased over time, with POC3 showing the highest number of unique fungal OTUs. This approach indicated that the microbial community adapted to changing fermentation conditions (He *et al*. 2015; Ganesan and Thiyagarajan 2025). Bacterial abundance peaked in POC1 and decreased through POC2 and POC3, reflecting bacterial adaptation to the changing fermentation environment (Chandna *et al*. 2013). In contrast, fungal abundance steadily increased with POC3 exhibiting the highest fungal diversity. This pattern reflected the known roles of bacteria in the early stages of fermentation, with fungi becoming more dominant in later stages when the decomposition of lignocellulose and other complex materials occurs (Huang *et al*. 2015).

Heatmap analysis confirmed these trends, showing that in POC1, genera such as *Lactobacillus*, *Pediococcus*, and *Streptococcus* were abundant, reflecting the dominance of lactic acid bacteria in the early stages of fermentation (Tran *et al*. 2019). Meanwhile, *Pichia*, *Penicillium* and *Hanseniaspora* emerged as dominant beneficial genera present in POC3. These genera are key players in the breakdown of complex organic materials and have roles in nutrient release and lignocellulose degradation, which played a critical role in determining the nutrient profile of LOF (Goh *et al*. 2020; Xu *et al*. 2022b; Gu *et al*. 2023). This shift from bacterial to fungal dominance underpins the microbial processes essential for the transformation of organic matter into nutrient-rich fertilizer.

Diversity indices for both bacteria and fungi further supported these findings. An increase in the Shannon and Simpson indices increased over time in bacterial analysis, indicating greater microbial diversity and evenness by POC3, while the Chao1 and ACE indices decreased, suggesting a more specialized bacterial community. For fungi, the Shannon index increased, and the Simpson index showed a decrease at POC2, followed by an increase at POC3, indicating a rise in fungal diversity and richness as fermentation progressed (Feranchuk *et al*. 2018).

While beneficial fungal genera such as *Penicillium*, *Pichia* and *Hanseniaspora* were present at the later stages of fermentation, the presence of potentially plant pathogenic genera, including *Colletotrichum* and *Gibberella*, in POC3 warranted further investigation (Masimbula *et al*. 2019; Gunasekaran *et al*. 2021). The detection of these genera highlighted the need for pathogen management strategies to ensure the safety and effectiveness of LOF as a fertilizer (Pornsuriya *et al*. 2023).

The microbial dynamics and nutrient transformations observed during LOF fermentation played a critical role in optimizing the production of nutrient-rich fertilizers. The shift from bacterial to fungal dominance, coupled with the transformation of organic waste into valuable nutrients, highlighted the importance of microbial processes in the sustainable production of LOF. However, further research is needed to explore the potential risks of pathogenic microorganisms and to optimize fermentation conditions to improve the quality and safety of LOF for agricultural use.

## Conclusion

Significant shifts in both microbial communities and nutrient profiles were observed throughout the fermentation process. The C/N ratio decreased over time, indicating the utilization of organic carbon by microbial communities, while N, P, and K content increased. Transformation of organic matter into plant-available nutrients supported the effectiveness of LOF as a nutrient source for crops. The microbial succession was evident, with bacteria dominating the early fermentation stages, followed by a shift towards fungal dominance, particularly members of Ascomycota and Basidiomycota, in

the later stages of fermentation. Results revealed crucial role of microbial dynamics in shaping the nutrient composition of LOF, which is vital for optimizing the fermentation process and enhancing the sustainability of LOF as an eco-friendly alternative to chemical fertilizers. This research contributed to the understanding of organic waste fermentation, demonstrating the potential of LOF to reduce environmental pollution while providing a sustainable fertilizer option for agriculture. Future research should focus on strategies to control potential pathogens during fermentation, ensuring the safety of LOF and exploring optimization of fermentation conditions to enhance nutrient content and microbial diversity.

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## Author Contributions

S conceptualized the study, designed the experiment, supervised the research, and led manuscript writing. BO collected samples, conducted nutrient and microbial analyses, and contributed to the manuscript. AR assisted in data analysis, especially metagenomic analysis, and helped interpret results. LDR contributed to the literature review, methodology, and provided insights into microbial dynamics. TDP supported fieldwork, data collection, experimental setup, and manuscript revisions. All authors approved the final manuscript

## Conflict of Interest

All authors declare no conflict of interest.

## Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

## Ethics Approval

Not applicable to this paper.

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