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Microbial and enzymatic dynamics during kitchen waste vermicomposting with contrasting carbon-rich substrates: Implications for biodegradation and quality

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Abstract

In sub-Saharan African cities, managing the growing volume of kitchen waste remains a critical challenge. This study evaluated the microbial and enzymatic profile during vermicomposting of organic kitchen waste (OKW) using the African earthworm *Eudrilus eugeniae*, specifically testing the impact of three carbon-rich amendments: grass clippings, sawdust, and cardboard waste. Conducted at the University of Yaounde I (Cameroon), the 60-day experiment compared five treatments (T0-, T0+, T1, T2, T3) to track microbial dynamics and the activity of five key enzymes (amylase, cellulase, protease, urease, and alkaline phosphatase) across three phases (Day 0, Day 30, and Day 60.). Morphological and biochemical identification revealed three species of bacteria, *Azotobacter* sp., *Bacillus* sp., and *Actinomyces*sp.; three species of fungi (*Fusarium* sp., *Penicillium* sp. and *Aspergillus* sp.) and three faecal coliform strains (*Klebsiella* sp., *Escherichia coli*. and *Enterobacter*sp.). The total coliform counts fell below WHO safety thresholds (< 1,000 CFU g^{-1}) in all treatments by day 60, validating the proces's sanitation efficiency. Enzymatic kinetics varied significantly by phase: urease peaked during pre-composting, protease at day 30, while amylase, cellulase, and phosphatase reached maximum activity at maturity (day 60). Cardboard (T3) and sawdust (T2) amendments produced the most distinct enzymatic signatures, with T3 showing the highest cellulase activity (7208.65 ± 57.53 µg/g dry weight glucose). These findings indicate that the choice of carbon amendments significantly modulates microbial structure and enzymatic speed, directly influencing the biodegradation rate and the final quality of the vermicompost.

Highlights

- Efficient kitchen waste recovery through vermicomposting using *Eudrilus eugeniae*
- Total coliform counts reduced to below WHO thresholds (<1,000 CFU/g) within 60 days
- Enzymatic peaks identified for urease (D0), protease (D30), cellulase/phosphatase (D60)
- Cardboard (T3) and sawdust (T2) specifically optimize cellulase and amylase kinetics
- Microbial diversity and enzymatic synergy ensure a mature, nutrient-rich vermicompost

1. Introduction

Rapid urbanisation and population growth in sub-Saharan Africa have led to a dramatic increase in municipal solid waste generation. Conventional disposal methods, including open dumping and incineration, are inadequate to address this challenge and pose serious environmental and public health risks [1, 2]. Vermicomposting is widely recognised as a sustainable and effective biotechnology for the stabilisation and recovery of various organic wastes, including food waste, agricultural by-products, sewage sludge, and manure [3, 4]. This process is facilitated by the synergistic interaction between earthworms and a diverse community of microorganisms, leading to the biological oxidation, decomposition, and humification of organic matter into a stable product known as vermicompost [5, 6].

The vermicompost produced in this process represents a nutrient-dense organic amendment capable of promoting soil fertility and plant productivity [7, 8, 9, 10]. Nevertheless, to fully qualify it as a high-quality organic amendment, rigorous maturity assessments, specifically the Germination Index (GI) are required to guarantee that the final product is free from phytotoxicity [11, 12]. The core of the vermicomposting process is the metabolic activity of microorganisms and the associated enzymatic systems.

Recent studies have shown that microbial communities (bacteria, fungi, and actinomycetes) play a central role in the degradation of complex organic compounds such as cellulose, hemicellulose, lignin, proteins, and lipids through the secretion of extracellular enzymes [13, 14, 15]. These enzymes, which include cellulases, proteases, lipases, amylases, ureases, and phosphatases, work as biocatalysts that accelerate the breakdown of organic matter and the mineralisation of nutrients, thereby improving the efficiency and stability of the vermicomposting process [16, 17, 18, 19]. Enzymatic activities are therefore widely regarded as reliable indicators of the biological maturity and agronomic quality of vermicompost [20, 21]. Studies have found that certain microbial taxa selectively proliferate during this process and contribute to increased enzymatic activity as well as the transformation of organic matter [22, 23]. This succession of microorganisms is closely linked to the composition of the substrate and the environmental conditions, which highlights the importance of interactions between microorganisms and enzymes for the efficiency of the process [24, 25] Earthworms play a complementary and essential role by modifying both the physical structure and the biological activity of the substrate. Through ingestion, fragmentation, and excretion, they increase the surface area available for microbial colonisation and stimulate microbial growth [13, 14]. The earthworm's gut significantly acts as a micro-reactor in which ingested microorganisms are selectively activated, contributing to enhanced enzymatic degradation [26]. The castings produced by earthworms are therefore rich in active microorganisms and enzymes, which further accelerate the stabilisation of organic matter [14, 27, 21]. Beyond earthworm biology, the nature of the feedstock substrate particularly its carbon-to-nitrogen (C:N) ratio and lignocellulosic content is a key determinant of microbial community structure and enzymatic activity during vermicomposting [28, 29]. Carbon-rich amendments such as sawdust, grass clippings, and cardboard waste differentially modulate fungal proliferation, cellulolytic activity, and nitrogen mineralisation. Yet comparative studies examining their simultaneous effects under tropical conditions remain scarce [20]. Despite the growing body of literature on vermicomposting, several critical knowledge gaps persist. First, most published studies have been conducted in temperate regions using European or North American earthworm species, and data on the African species *Eudrilus eugeniae* under tropical climatic conditions remain limited [10]. Second, while the individual effects of carbon amendments on vermicomposting have been reported, comparative studies simultaneously evaluating the influence of contrasting carbon sources with varying in C:N ratio, lignin content, and physical structure on both microbial community dynamics and enzymatic activity profiles are scarce. Third, the sanitation efficiency of vermicomposting with respect to faecal coliform reduction in kitchen waste from urban sub-Saharan African contexts has received little systematic attention, despite its direct relevance to public health and compost quality standards. Addressing these gaps is essential for developing

locally adapted, cost-effective vermicomposting systems suited to the organic waste management challenges of rapidly growing African cities.

The following theories have been developed in light of the aforementioned: (H1) During vermicomposting, the type of carbon amendment greatly affects the microbial community structure and enzymatic activity profiles. Lignocellulose-rich substrates (cardboard, sawdust) encourage different fungal proliferation and cellulolytic activity in comparison to nitrogen-rich grass clippings. (H2) In line with the succession of organic matter decomposition, the combined activity of *Eudrilus eugeniae* and related microbial communities causes a progressive temporal shift in enzymatic activities (from urease and protease dominance in early stages to amylase, cellulase, and phosphatase dominance at maturity).

The present study therefore aimed to characterize the temporal dynamics of five key enzymes and microbial communities (bacteria, actinomycetes, fungi, and faecal coliforms) during the vermicomposting of organic kitchen waste amended with three contrasting carbon sources using *Eudrilus eugeniae* under tropical conditions in Yaounde, Cameroon.

2. Materials and Methods

2.1. Study site

The research was conducted at the University of Yaounde I (Cameroon 3° 51‘ 30” N,11° 29’ 30” E). Yaounde, the capital city of Cameroon, has a humid tropical climate characterised by high mean annual temperatures (23-25°C) and two rainy seasons**.** The experiment was conducted during the major rainy season from June to September 2023 (corresponding to conditions of high ambient humidity and moderate temperature).

2.2. Organic waste collection and preparation

The organic waste was collected from private households in the city of Yaounde and from university restaurants on the campus of the University of Yaounde I. It was manually sorted to eliminate plastics, broken glass, and any non-biodegradable waste. The sorted waste was weighed into piles of 75 kg and mechanically reduced to small pieces (≤5 cm) to increase the contact surface available for microbial colonisation. Carbon-rich additives (dry grass clippings, sawdust, and cardboard waste) were added at a mass ratio of 1:3 (amendment: kitchen waste, w/w), corresponding to 25 kg of amendment per 75 kg of kitchen waste, with the exception of the control treatment (T0-). Each pile was thoroughly mixed, moistened to approximately 60-70% moisture content, and covered with plastic sheeting to maintain aerobic conditions for the pre-composting process. Temperature within each pile was monitored once every three days using a compost thermometer to confirm thermophilic activity (≥ 45°C). After 21 days of pre-composting, representative samples (≈500 g composite from five sub-samples per pile) were collected in sterile, sealed containers and transported to the laboratory at 4°C for analysis.

2.3. Experimental Setup and Vermicomposting

Eudrilus eugeniae earthworms were obtained from the vermiculture station of the University of Yaounde I. Prior to the experiment, earthworms were acclimatised for seven days in pre-composted kitchen waste to reduce handling stress. Adult individuals were selected for uniformity. After 21 days of pre-composting, the precomposts were distributed in ventilated wooden boxes measuring 45 cm×45 cm×45 cm and 45 cm deep, lined with fine mesh netting (1 mm) to prevent earthworm escape while maintaining aeration. Each box received 10 kg of pre-compost, placed under a shade shelter to minimise direct solar radiation and extreme temperature,and waswatered twice weekly to maintain substrate moisture at 60-70%.

Five treatments were established in triplicate (n = 3 replicates per treatment), as follows:

- T0- = organic kitchen waste

- T0+ = organic kitchen waste + 350 earthworms

- T1 = organic kitchen waste + dry grass clippings + 350 earthworms

- T2 = organic kitchen waste + sawdust + 350 earthworms

- T3 = organic kitchen waste + cardboard waste + 350 earthworms.

The earthworm density of 350 individuals per box (**3,46 kg/m²**) is consistent with established protocols for *Eudrilus eugeniae* vermicomposting studies [30]. Vermicomposting lasted 60 days, during which the enzymatic activity of five enzymes (amylase, Cellulase, protease, urease, and alkaline phosphatase) was analysed at three different phases: Day 0 (pre-compost), Day 30, and Day 60.

2.4. Microbial diversity

The microbial load of the various samples was determined by serial tenfold decimal dilution (10^{-1} to 10^{-8}) in sterile peptone water, and the colony count was converted to CFU g^{-1} fresh weight using the following formula [31] :

$$C(CFU/g) = \frac{\text{Number of colonies counted x dilution factor}}{\text{Volume inoculum applied (0.1 mL)}}$$

Bacterial isolates were identified based on macroscopic, microscopic, and biochemical characteristics as described by [32]; [33] with reference to the Bergey's Manual of Systematics of Archaea and Bacteria [34].

Fungal isolates were identified based on morphological criteria of the culture or hyphae, spore characteristics, and reproductive structures [35, 36]

The microbial dynamics were assessed using culture-based techniques; however, it must be acknowledged that plate counting often underestimates the actual microbial diversity compared with

modern molecular methods like 16S/ITS sequencing [22].

2.5. Enzymatic analysis

2.5.1. Preparation of enzyme extracts and substrate solutions

Samples of 100 g were collected from each treatment at each sampling time (Day 0, Day 30, Day 60), placed in plastic bags, and kept at 4°C until analysis (within 24 h of collection). Enzyme extracts were prepared from 5 g of fresh sample, homogenised in a mortar with liquid nitrogen to prevent protein denaturation. The homogenate was resuspended in 10 mL of 0.1 M potassium phosphate buffer (pH 7.0) containing 0.5 M NaCl in 50 mL conical tubes and vortexed for 2 min. The mixtures were centrifuged at 3000 rpm for 5 min to remove any residues. The resulting supernatant was used as the crude enzyme extract. Substrate solutions were prepared as follows: starch (5%, w/v) for amylase; carboxymethylcellulose (CMC, 5%, w/v) for cellulase; urea (5%, w/v) for urease; and bovine serum albumin (BSA, 1%, w/v) for protease. The substrate for phosphatase was p-nitrophenyl phosphate (pNPP, 38 mmol)

2.5.2. Measurement of enzymatic kinetics

All enzymatic reactions were carried out under standardized conditions: incubation at 37°C for 2 h, followed by colorimetric quantification based on Beer–Lambert's law. Absorbance was measured using a DR/3900 spectrophotometer. A 1 mL volume of supernatant was supplemented with one drop of toluene to inhibit residual microbial activity during incubation.

For cellulase and amylase activities, 5 mL of the respective substrate solutions of carboxymethylcellulose (CMC, 5%) and starch (5%) were added to the enzyme extract. After homogenisation and incubation, absorbance was measured at 510 nm. Enzyme activities were expressed as µg of glucose released per gram of fresh weight (FW).

Urease activity was determined by adding 100 µL of extract to 1 mL of urea reagent. After homogenisation and incubation under the same conditions, absorbance was measured at 578 nm. The results were expressed as mg of NH_4^+ per gram of FW.

Protease activity was determined using a 1% albumin solution as the substrate. A 400 µL aliquot of extract was mixed with the albumin solution, followed by the addition of 100 µL of Folin's reagent. After incubation under the same conditions, the absorbance was measured at 700 nm. Activity was expressed as µg tyrosine equivalents g^{-1} FW, using a standard curve prepared with L-tyrosine (0-500 µg mL^{-1}) [37].

Finally, alkaline phosphatase activity was determined using an optimised DEA DGKC colorimetric method. 800 µL of diethanolamine buffer (pH 9.8) was added to 100 µL of sample and 200 µL of p-nitrophenyl phosphate (38 mM). Absorbance was measured at 405 nm at three time points: 1 min, 2 min, and 3 min. The rate of *p*-nitrophenol (pNP) release was calculated from the linear portion of the

absorbance-time curve using a pNP standard curve (0-200 µg mL^{-1}). Alkaline phosphatase activity was expressed as µg of p-nitrophenol per gram of FW.

2.6. Statistical analysis

All data are expressed as the mean ± standard deviation (SD) of three independent replicates. Data were checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) prior to analysis. When assumptions were met, a one-way analysis of variance (ANOVA) was performed using RStudio (version 4.0.5; R Core Team, 2021). Where ANOVA indicated significant differences ($p < 0.05$), means were compared using Duncan's multiple-range test at the 5% significance level. Figures were produced using GraphPad (version 8.0.1). All statistical tests were conducted at $\alpha = 0.05$.

3. Results and Discussion

3.1. Microbial dynamics

Microbial load varied significantly over time ($p < 0.05$) across all treatments (Table 1), under the combined influence of treatment type, substrate characteristics, and earthworm biological activity. Overall, bacterial populations, actinomycetes, and total coliforms decreased significantly ($p < 0.05$) between the pre-composting stage and mature vermicompost for all treatments, whereas fungal populations increased steadily over time. A clear sequence of microbial succession was observed. The temporal succession of microbial communities observed in this study followed a pattern broadly consistent with those reported in the literature for *Eudrilus eugeniae* and other epigeic species [38]. The initial proliferation of bacteria and actinomycetes during the active phase (Day 0–30) reflects intense mineralisation of readily degradable substrates (carbohydrates, proteins) driven by earthworm mediated fragmentation, aeration, and microbial inoculation [14, 15]. The subsequent decline (Day 30–60) is consistent with substrate depletion and increased interspecific microbial competition [27], and aligns with the shift toward humification that characterises vermicompost maturation [26]. Bacterial populations, actinomycetes, and fungi initially increased between the pre-composting stage (Day 0) and the intermediate stage (Day 30), indicating an active phase of microbial proliferation facilitated by the presence of readily degradable substrates. Subsequently, a marked depletion of these microbial groups was observed between Day 30 and Day 60 (maturity stage). The type of carbonaceous amendment had a significant effect ($p < 0.05$) on microbial populations (Table 1), most markedly at the intermediate stage (Day 30). The contrasting dynamics of fungal populations (which increased throughout the process, particularly at maturity) are ecologically significant. Fungi are key degraders of recalcitrant lignocellulosic compounds [39, 40], and their proliferation in the late stage reflects the progressive dominance of complex carbon substrates as labile fractions are exhausted. This pattern supports hypothesis H2: the temporal shift in enzymatic activity (from urease/protease dominance to cellulase/phosphatase dominance) mirrors the compositional shift of active microbial communities from bacteria-dominated (early stage) to fungi-dominated (late stage) assemblages. Fungal abundance at Day 30 followed the order: T3 (1.04×10^8 CFU g^{-1}) > T2 (3.03×10^7) ≈ T0+ (2.90×10^7) > T1 ($1.47 \times$

10^7) ≈ T0- (1.13×10^7). Relative to T0-, fungal populations increased, demonstrating that cardboard waste strongly stimulated fungal proliferation during the active decomposition phase. Bacterial abundance was significantly higher in T1 (1.29×10^{11} CFU g^{-1}) and T2 (1.39×10^{11} CFU g^{-1}) compared to T0-, T0+, and T3 at Day 30. Relative to T0-, bacterial counts increased in T0+, T1, T2, and T3. The strong stimulating effect of cardboard waste (T3) on fungal proliferation at Day 30 (+ 920% relative to T0-) can be attributed to its very high C:N ratio (≈ 35:1) and elevated cellulose content, which selectively favoured cellulolytic and ligninolytic fungal taxa such as the *Penicillium* and *Aspergillus* genera identified here [22, 41]. By contrast, the higher bacterial abundances in T1 and T2 (grass clippings and sawdust) are likely attributable to their more balanced C:N ratios, which provided more readily available nitrogen for bacterial growth, consistent with findings on nitrogen-mediated bacterial stimulation in organic waste composting [28, 29]. These results provide partial support for the hypothesis that carbon amendment type significantly modulated microbial community structure, although the direction of the effect differed between bacteria (stimulated by low C:N amendments) and fungi (stimulated by high C:N amendments).

A progressive decrease in total coliform populations was observed throughout the process. At the pre-composting stage, coliform levels were significantly lower in the amended treatments. At the intermediate stage, T1, T2, and T3 showed additional reductions, respectively, compared with T0-. At maturity (Day 60), total coliform counts ranged from 2.50×10^2 to 2.47×10^3 CFU g^{-1}, below the threshold of < 1,000 CFU g^{-1} for compost applied to fruit and vegetable crops and < 5,000 CFU g^{-1} for cereal crops. All treatments achieved total coliform counts below WHO [42] safety thresholds at Day 60 (< 1,000 CFU g^{-1} for fruit/vegetable crops; < 5,000 CFU g^{-1} for cereals), indicating that vermicomposting with *Eudrilus eugeniae* effectively reduces specific bacterial loads across different carbon amendments, thereby improving microbiological safety, although it does not eliminate all pathogenic risks, like helminth eggs, according to [43]. These results are consistent with those of [44]and [45]), who demonstrated that earthworms reduce faecal coliform populations through multiple mechanisms, including gut passage, antimicrobial coelomic secretions, and the stimulation of antagonistic microbial communities. The earlier and more pronounced reductions observed in amended treatments (T1-T3) at the pre-composting and intermediate stages suggest that the presence of carbon-rich substrates accelerates sanitation, possibly by promoting competitive exclusion of coliforms by rapidly proliferating cellulolytic bacteria and fungi [46]. These findings have significant implications for waste management systems in municipalities across sub-Saharan Africa, where kitchen waste often contains high levels of faecal coliforms due to inadequate sanitation infrastructure; the strategic incorporation of locally available carbon-based amendments (sawdust or cardboard from markets) could effectively improve both the biosafety of the process and the final quality of the compost.

Table 1
Microbial load during vemicomposting

Stage	Treatments	Bacterial populations (CFU g^{-1})	Actinomycetes (CFU g^{-1})	Fungal populations (CFU g^{-1})	Total Coliforms (CFU g^{-1})
Pre-compost (D0)	T0-	3.40×10^{8} c	2.20×10^{6} d	2.60×10^{5} f	8.70×10^{6} a
	T0+	3.40×10^{8} c	2.20×10^{6} d	2.60×10^{5} f	8.70×10^{6} a
	T1	2.80×10^{8} c	4.60×10^{6} d	9.70×10^{5} ef	4.60×10^{6} bc
	T2	2.60×10^{8} c	2.20×10^{6} d	2.40×10^{5} f	4.30×10^{6} c
	T3	2.60×10^{8} c	3.60×10^{6} d	2.20×10^{5} f	5.20×10^{6} b
Mid stage (D30)	T0-	4.83×10^{10} b	5.90×10^{7} c	1.13×10^{7} c	4.73×10^{6} bc
	T0+	5.67×10^{10} b	1.80×10^{8} b	2.90×10^{7} b	4.77×10^{6} bc
	T1	1.29×10^{11} a	2.17×10^{8} b	1.47×10^{7} c	3.40×10^{6} d
	T2	1.39×10^{11} a	3.97×10^{8} a	3.03×10^{7} b	3.27×10^{6} d
	T3	6.90×10^{10} b	2.03×10^{8} b	1.04×10^{8} a	3.03×10^{6} d
Mature vermicompost (D60)	T0-	2.47×10^{6} c	4.17×10^{4} d	5.37×10^{6} de	2.47×10^{3} f
	T0+	9.03×10^{5} c	3.57×10^{4} d	6.57×10^{6} d	1.12×10^{3} f
	T1	7.33×10^{5} c	2.43×10^{4} d	2.23×10^{6} def	2.50×10^{2} f
	T2	5.20×10^{5} c	2.77×10^{4} d	3.93×10^{6} def	2.77×10^{2} f
	T3	4.77×10^{5} c	2.10×10^{4} d	3.23×10^{6} def	3.53×10^{2} f
	Probability (p)	9.89e-15 ***	< 2e-16 ***	< 2e-16 ***	< 2e-16 ***

T0-: organic kitchen waste; T0+: organic kitchen waste + earthworms; T1: organic kitchen waste + grass clippings + earthworms; T2: organic kitchen waste + sawdust + earthworms; T3: organic kitchen waste + cardboard waste + earthworms. For each parameter, the means of treatments denoted by the same letters are not significantly different at the 5 per cent significance level of Duncan's test.

3.2. Microbial diversity

Morphological and biochemical characterisation (Figs. 1 and 2) identified three faecal coliform genera: *Escherichia coli* and *Enterobacter sp*., and *Klebsiella* sp., which are standard indicators of faecal contamination [42].

Among beneficial bacteria, *Azotobacter* sp., *Bacillus* sp., and *Actinomyces* sp. were identified in all treatments at all three sampling stages. Among fungi, *Fusarium* sp., *Aspergillus* sp., and *Penicillium* sp. were consistently detected, with increasing abundance towards maturity (Fig. 2). All microbial identifications are presumptive, based on morphological and biochemical criteria; molecular confirmation was beyond the scope of this study. The identification of *Azotobacter* sp., *Bacillus* sp., and *Actinomyces* sp. as the dominant bacterial genera is consistent with previous vermicomposting studies [47, 48, 49] and supports the biofertilizer potential of the vermicompost produced. These taxa contribute to nitrogen fixation (*Azotobacter*), hydrolytic enzyme production (*Bacillus*), and complex polymer degradation (*Actinomyces*). These functions are directly relevant to the agronomic quality of vermicompost [17, 50].

The detection of *Fusarium sp., Aspergillus sp., and Penicillium* sp. reflects the progressive enrichment of the fungal community with lignocellulosic degrading taxa, consistent with recent studies [22, 41]. The capacity of *Aspergillus* spp. to solubilize inorganic phosphorus [40] is particularly relevant in the context of the high alkaline phosphatase activities recorded at Day 60 in T1 and T3, suggesting a functional link between *Aspergillus* proliferation and phosphate mobilisation in these treatments. However, it should be noted that *Fusarium* spp. include phytopathogenic strains, and the agronomic safety of vermicompost containing this genus should be assessed in future studies.

3.3. Enzymatic activity

3.3.1. Amylase activity

Amylase activity followed a biphasic pattern throughout the vermicomposting process, increasing markedly during the active decomposition phase (Days 0–30) before declining as the substrate matured (Fig. 3A). During the first 30 days, enzyme activity increased significantly in all treatments ($p < 0.05$), except in T0+, where the increase was not statistically significant ($p = 0.12$). The strongest responses were observed in T2 and T0-, with increases of 350.4% and 312.5%, respectively, while T1 and T3 showed more moderate but still significant increases of 146.5% and 126.0%.

During the maturation phase, a consistent decline was observed across all treatments, reflecting the progressive depletion of easily degradable carbohydrates. The largest decreases occurred in T2 (69.7%)

and T0- (52.4%), whereas T1 showed the smallest reduction (14.9%), indicating more sustained amylolytic activity during the later stages of vermicomposting. By Day 60, the highest amylase activities were still recorded in T2 (2,330.44 ± 188.43 µg glucose g^{-1} FW) and T0- (2,223.02 ± 146.68 µg glucose g^{-1} FW).

The temporal trend observed for amylase closely resembled that of protease, with both enzymes reaching maximum activity around Day 30 before declining during maturation. The trend reflects the rapid utilization of readily biodegradable substrates, particularly starch and proteins, which are abundant in the early stages of kitchen waste decomposition. The high amylase activity recorded in T2 and T0- during the active phase is therefore likely related to the starch-rich composition of the feedstock, including potato, cocoyam, and yam peels, which provide an easily accessible carbon source for amylolytic microorganisms. Similar increases in amylase activity during the early stages of vermicomposting have been reported by [20] and [51], who associated this response with intensified microbial carbon metabolism.

The relatively stable amylase activity observed in T1 during the maturation phase may be explained by the presence of grass clippings, which likely promoted a more gradual carbon-to-nitrogen equilibration. This slower stabilisation process may have prolonged the availability of degradable carbohydrates, allowing amylolytic microorganisms to remain active for longer than in the other treatments. Overall, the observed dynamics indicate that amylase activity is closely linked to substrate quality and can serve as a useful indicator of the transition from active organic matter decomposition to vermicompost maturation.

3.3.2. Cellulase activity

Cellulase activity displayed a characteristic V-shaped pattern throughout the 60 day vermicomposting process (Fig. 3B). During the active decomposition phase (Days 0–30), enzyme activity declined in all treatments, with the greatest reductions observed in T2 (66.0%) and T1 (44.7%), whereas the control without earthworms (T0-) showed only a slight decrease (4.4%). This trend was reversed during the maturation phase (Days 30–60), when cellulase activity increased markedly across all treatments. The most pronounced recovery occurred in T2, where activity increased by 247.3%, while T1, T3, and T0 + also showed increases exceeding 100%. These recovery rates differed significantly from those of the mature control by Day 60 ($p < 0.05$). At the end of the experiment, T3 exhibited the highest cellulase activity (7,208.65 ± 57.53 µg glucose g^{-1} FW), followed by T0 + and T0-. Interestingly, cellulase activity in the control treatments exceeded that measured in T1 and T2, despite the addition of cellulose-rich materials such as grass clippings and sawdust in the latter treatments.

The similar evolution of cellulase activity has been reported previously as a common feature of vermicomposting [20, 52]. The initial decline likely reflects a temporary reduction in cellulolytic activity during the early stages of decomposition. One possible explanation is that earthworms' diet alters the fungal populations responsible for breaking down cellulose, thereby reducing cellulase production. However, because the present study relied on culture-based microbiological methods, this hypothesis

cannot be confirmed directly. High-throughput molecular approaches, such as ITS sequencing, would provide a more comprehensive assessment of fungal community dynamics during vermicomposting.

The strong recovery observed by Day 60 is consistent with the maturation of the substrate. As easily degradable organic compounds become depleted, microorganisms increasingly rely on more resistant lignocellulosic materials, stimulating renewed cellulase production. At the same time, continued fragmentation of the substrate by earthworms improves the accessibility of cellulose embedded within complex plant tissues, facilitating microbial degradation of these recalcitrant fractions [29].

The relatively lower cellulase activities measured in T1 and T2 at maturity may seem unexpected given their enrichment with cellulose-rich amendments. However, the presence of abundant cellulose does not necessarily translate into greater enzyme activity. In particular, the highly recalcitrant structure of sawdust may limit microbial access to cellulose because of its close association with lignin, thereby reducing the efficiency of cellulolytic microorganisms despite the high cellulose content [29]. This interpretation may explain why T2 did not achieve the highest cellulase activity at the end of the process. In contrast, T3 maintained a high cellulase activity at maturity, consistent with the findings of [28], who reported enhanced cellulose degradation when lignocellulosic substrates possess physicochemical characteristics that favour microbial colonisation and enzymatic hydrolysis.

3.3.3. Urease activity

Urease activity decreased significantly throughout the 60-day vermicomposting process ($p < 0.05$) in all treatments (Fig. 3C), indicating a gradual reduction in nitrogen transformation activity as decomposition progressed. During the active phase (Days 0–30), urease activity decreased by 52.3% in T1 and up to 60.1% in T2, with no significant differences among treatments. The decline continued during the maturation phase (Days 30–60) in all treatments containing earthworms, eventually reaching relatively stable levels by the end of the experiment.

An interesting observation was made in the control treatment without earthworms (T0-), where urease activity increased significantly between days 30 and 60 ($p < 0.05$). This contrasting response suggests that earthworms may influence nitrogen transformations during vermicompost maturation by regulating microbial urease activity. One possible explanation is that earthworms promote nitrogen immobilisation within their biomass or alter the availability of ammonium (NH_4^+), thereby modifying the feedback mechanisms that control urease production. Another possibility is that earthworm activity favours nitrifying microbial communities, which may compete with ureolytic microorganisms and reduce urease expression. Although these mechanisms remain to be fully clarified, they point to a specific role of earthworms in regulating nitrogen cycling during vermicomposting.

The overall decrease in urease activity is consistent with previous studies showing that, as readily available nitrogen compounds are consumed, ammonium accumulates and exerts inhibitory effects on urease synthesis and activity [53, 54]. Consequently, lower urease activity is generally associated with substrate stabilisation and the transition from active decomposition to compost maturation. In this

regard, the low and relatively stable urease activities recorded by Day 60 in all earthworm amended treatments are consistent with the observations of [54], who proposed urease activity as a useful biochemical indicator of vermicompost maturity. These results therefore suggest that the active bioconversion phase had largely ended by Day 60, confirming the progression of the substrate toward a stable and mature vermicompost.

3.3.4. Protease activity

Protease activity followed a characteristic bell-shaped pattern throughout the vermicomposting process in all treatments (Fig. 3D), increasing rapidly during the active decomposition phase before declining as the substrate matured. Enzyme activity rose significantly in T0-, T0+, T1, and T2, reaching levels five to six times higher than their initial values, whereas T3 showed a more moderate response, with activity approximately doubling over the same period. During the maturation phase (Days 30–60), protease activity declined across all treatments. The largest decreases were observed in T0- (39.78%) and T0+ (38.20%), while T1 remained comparatively stable, showing only a 21% reduction.

The pronounced increase in protease activity during the early stages of vermicomposting can be attributed to the abundance of readily degradable protein substrates, which stimulate microbial growth and metabolic activity [54]. In addition, the passage of organic material through the earthworm digestive tract enriches the substrate with proteolytic enzymes produced by the gut microbiota, further enhancing protease activity in the resulting vermicompost [20]. Similar trend have been reported in previous vermicomposting studies. For example, [30] and [55] observed substantial increases in protease activity during the processing of municipal solid waste, reflecting intense microbial degradation of organic matter.

The decline in protease activity during the maturation stage is consistent with the progressive depletion of easily degradable nutrients and the stabilisation of the organic substrate [56]. As decomposition advances, the organic carbon content decreases, humification intensifies, and the vermicompost gradually reaches maturity. Under these conditions, microbial demand for proteolytic enzymes declines, resulting in lower enzyme activity. A similar trend was described by [57], who reported maximum protease activity around Day 30, followed by a gradual decrease until the end of the vermicomposting process, confirming that protease activity is closely linked to substrate availability and the stage of compost stabilisation.

3.3.5. Alkaline Phosphatase activity

Alkaline phosphatase activity changed significantly throughout the vermicomposting process ($p < 0.05$), with distinct temporal patterns among the treatments (Fig. 3E). During the active decomposition phase (Days 0–30), enzyme activity increased markedly in all treatments, reflecting an enhanced microbial demand for phosphorus as the organic matter was rapidly degraded. Peak activity was reached on Day 30 in treatments T0-, T1, and T3, after which a significant decline was observed during the maturation phase, with reductions of 70.0%, 90.0%, and 60.6%, respectively. In contrast, T0 + and T2 maintained an upward trend until Day 60, with modest increases of 9.7% and 31.9%, although these changes were not

statistically significant ($p > 0.05$). By the end of the experiment, the highest alkaline phosphatase activities were recorded in T1 (6,382.3 ± 250.37 µg pNP g^{-1} FW) and T3 (5,729.3 ± 233.36 µg pNP g^{-1} FW), highlighting their strong capacity for phosphorus mineralisation. Among all the enzymes evaluated, alkaline phosphatase displayed the most complex response, with activity patterns varying considerably according to treatment.

The initial rise in enzyme activity is consistent with previous studies reporting increased phosphatase production during the intensive phase of organic matter decomposition, when microbial communities require large amounts of phosphorus for growth and metabolism [58, 59]. The subsequent decline observed in T0-, T1, and T3 is likely explained by the accumulation of inorganic phosphate (Pi), which is known to suppress phosphatase synthesis through feedback inhibition [20]. Conversely, the sustained increase in T0 + and T2 suggests that phosphorus became available more gradually in these treatments. This delayed release may be associated with the physical retention of phosphate within earthworm casts in T0 + or its adsorption onto the sawdust matrix in T2, thereby limiting Pi accumulation and reducing feedback inhibition.

The particularly high alkaline phosphatase activity measured in T1 at Day 60 indicates a strong phosphate-solubilising potential, suggesting that the vermicompost produced under this treatment could provide a readily available source of phosphorus for plants. This characteristic enhances its agronomic value and supports its potential use as an efficient organic fertiliser.

4. Conclusion

This study demonstrates that vermicomposting effectively regulates cultivable microbial communities and enzymatic activities, producing a stable organic soil amendment with improved nutrient availability. The marked reduction in total coliforms indicates a substantial improvement in microbiological quality, although it should not be interpreted as complete sanitisation, particularly with respect to more persistent pathogens such as helminth eggs. It is also important to acknowledge that the culture-based methods used in this study capture only a fraction of the microbial community and therefore underestimate the overall microbial diversity compared with high-throughput molecular approaches such as 16S rRNA and ITS sequencing. Despite these limitations, the final vermicompost exhibits characteristics of a high-quality biofertiliser with considerable potential to enhance soil fertility and contribute to more sustainable agricultural practices.

Declarations

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Authorship contribution

Ginette Sandrine Liégui: Conceptualization, Methodology, Funding acquisition, field experiment, Data collection, Formal analysis, Writing Original Draft. **Temegne Nono Carine**: Data analysis, Writing – review. **Alain-Martial Sontsa- Donhoung**: Methodology, Writing – review. ***Jean Jules NdangangNana***: Data collection. **Wafo Djumyom**: Writing – review. **Emmanuel Youmbi :** Supervision, Validation. **Toguet Zébazé**: Supervision, Validation.

Declarations of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used **ChatGPT (OpenAI)** to assist with English synthesis and language refinement of the text, with the aim of improving clarity, coherence, and readability. The AI-generated suggestions were used solely to enhance the linguistic quality of the manuscript and did not contribute to the scientific content, interpretation of the results, or conclusions. All generated text was carefully reviewed, revised where necessary, and approved by the authors, who assume full responsibility for the accuracy, originality, and integrity of the final manuscript.

Data availability

The data will be shared upon reasonable request to the corresponding author.

Ethics approval and consent to participate

The collection of earthworms used in the study complies with national guidelines and requires no further affirmation.

Consent for publication

Not applicable.

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Figures

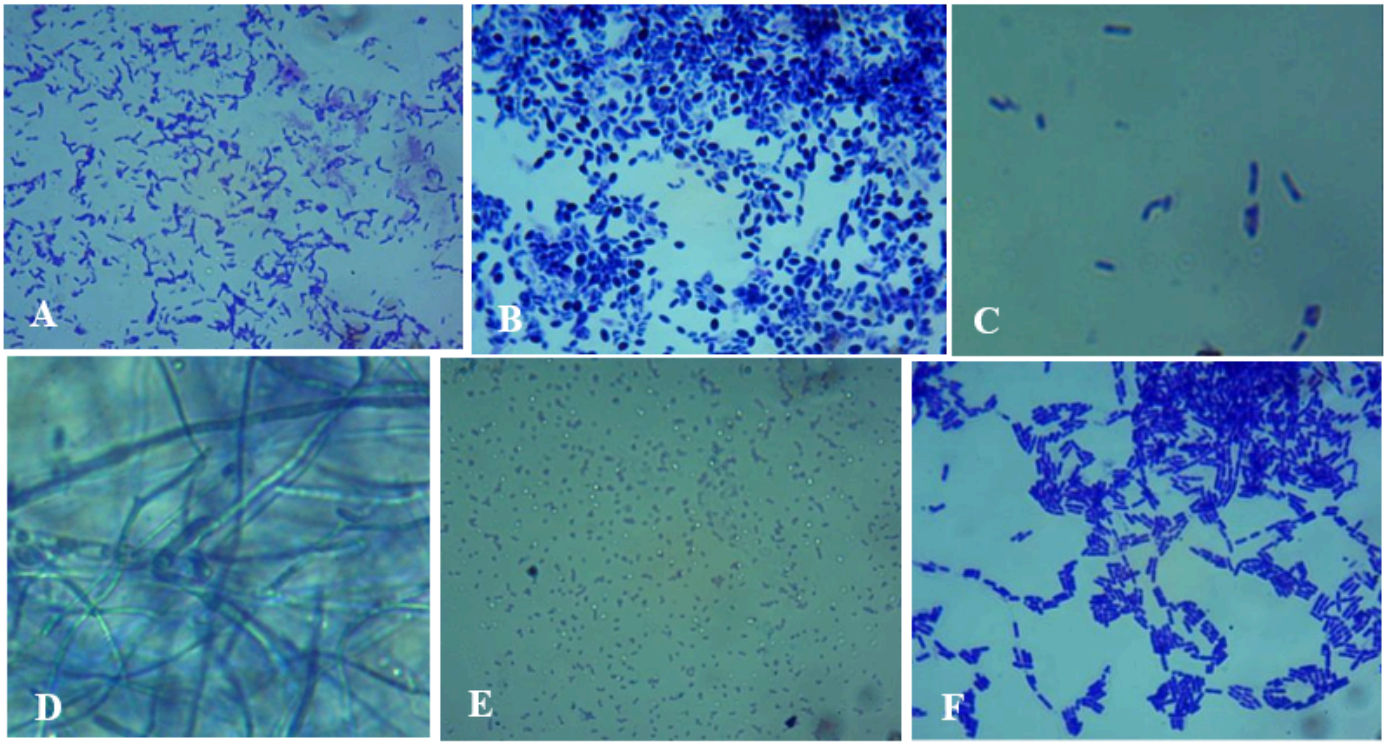


Figure 1

Microscopic isolated bacterial. (A: *Klebsiella* sp.; B: *Enterobacter* sp.; C: *Escherichia coli* ; D: *Actinomyces* sp. ; E: *Azotobacter* sp. ; F: *Bacillus*sp.)

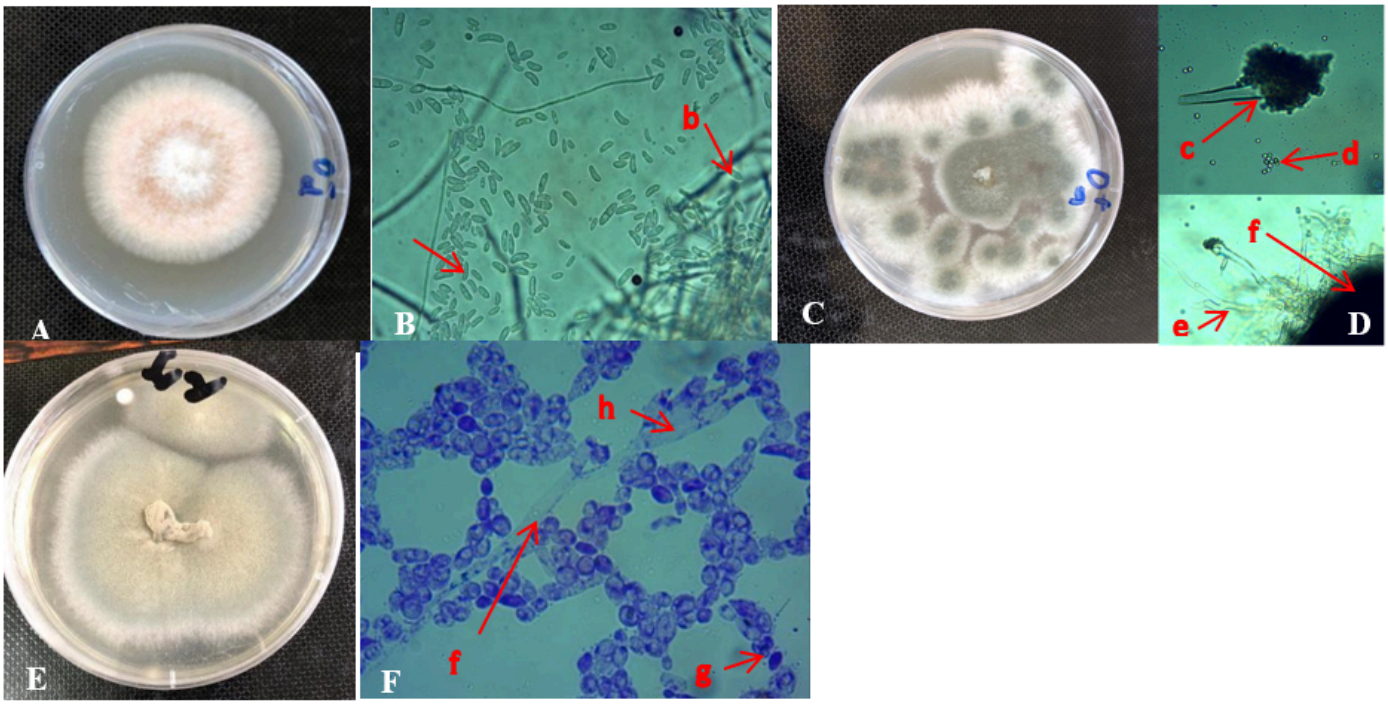


Figure 2

Morphological and microscopic characterization of isolated fungal strains. (A: Macroscopic structure of *Fusarium* sp. in a Petri dish; B: Microscopic structures of *Fusarium*sp. (a: conidia, b: hyphal aggregates) ; C: Macroscopic structure of *Aspergillus*sp. in a Petri dish; D: Microscopic structures of *Aspergillus* sp. (c: conidiophore with spore cluster, d: conidia, e: hyphae, f: phialide); E: Macroscopic structure of *Penicellium* sp. in Petri dish; F: Microscopic structures of *Penicellium* sp. (f: conidiophore, g: conidia, h: phialides))

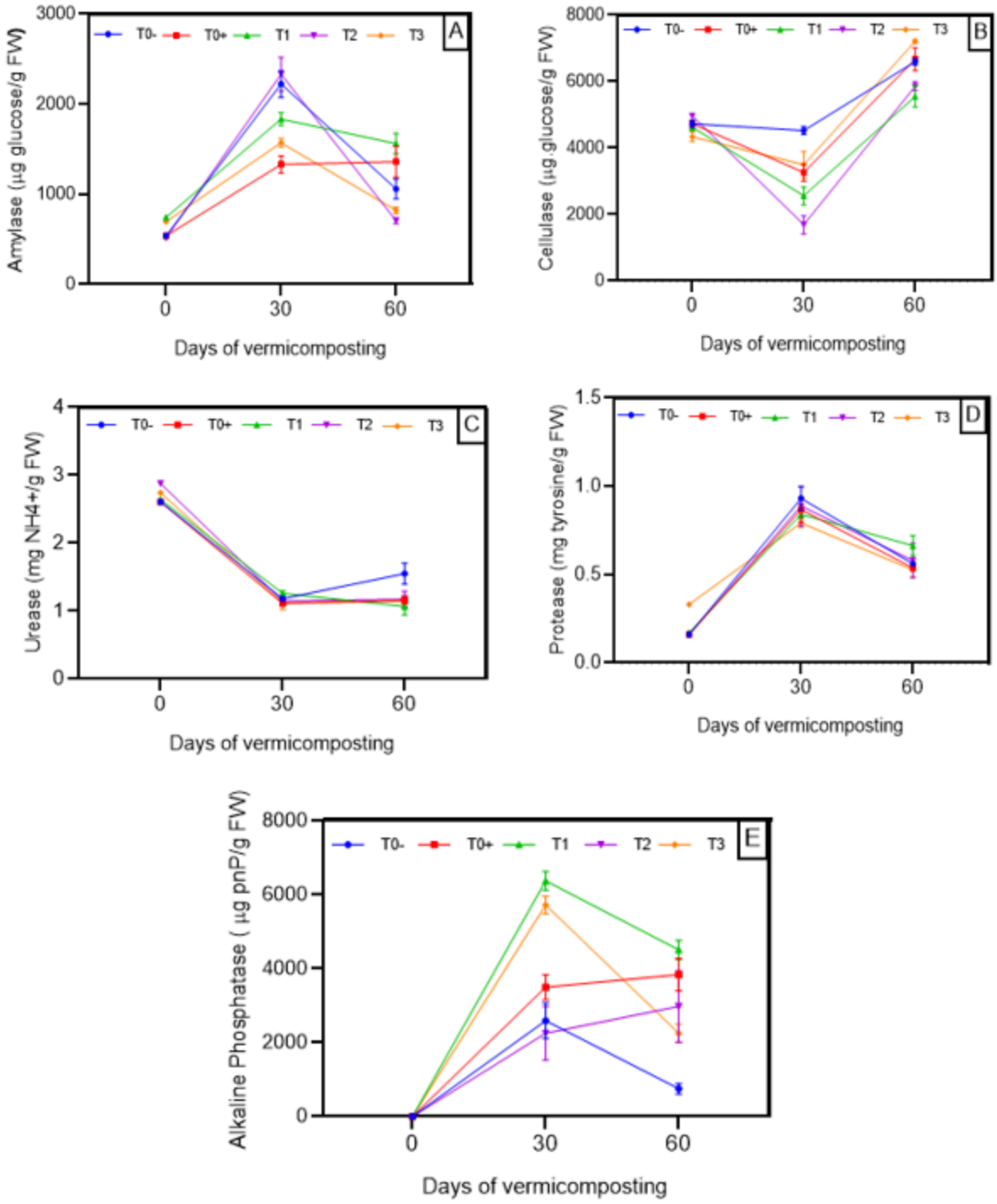


Figure 3

Enzymatic kinetics during vermicomposting.(A: Amylase, B: Cellulase, C: Urease, D: Protease, E: Alkaline phosphatase).