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Sustainable production of mature and stable amendments through biochar-enhanced vermicomposting of cocoa pod husks

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A R T I C L E I N F O

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A B S T R A C T

A major waste resulting from cocoa production is cocoa pod husk (CPH), which is often piled up on-farm and left to rot. This study aims to valorise CPH through a vermicomposting process to produce stable and mature amendments. Fresh CPH were mixed with cattle manure (ratio 1:1), to this mixture 0, 4 and 8 % CPH biochar were added. The earthworm species, *Eudrilus eugeniae*, which is native to the Ghana region and is readily accessible to local farmers, was used for the vermicomposting over a 60-day duration. Chemical parameters (pH, electrical conductivity, organic C, total N, P, K, Ca, Mg) and microbial characteristics (culturable bacteria and fungi and DNA sequencing) were monitored during the vermicomposting. The pH, total N and K of the different matrices raised over time and with increasing amounts of biochar. In all treatments, bacterial counts decreased in the first 10 days (between 1.79 and 2.16 times) and then stabilised throughout the process. Biochar inhibited the number of fungi in the first 40 days, but subsequently stimulated their growth. The biochar addition had a significant impact on the dynamics of the bacterial communities, although *Streptomyces, Pelagibacterium, Rhabdothermus, Lysinibacillus, Micromonospora,* and *Mesorhizobium* were the dominant genera in all samples. All treatments promoted an increase in microbial taxa involved in carbon and nitrogen cycle.

The results highlighted that CPH can be valorised through the production of vermichar, although scalability and efficiency assessments need to be performed, to ensure the applicability of this bioprocess on large scale in cocoa-growing regions.

1. Introduction

Agriculture alone produces more than 1.33 billion tonnes of organic wastes per year globally [1], with a constant increase driven by the continuous expansion of intensive agricultural activity in response to the growing human population [2]. Cocoa farming is one of the agricultural activities that produces high amounts of organic waste in the form of cocoa pod husks (CPH) [3]. Ghana alone generates about 858,720 tons of CPH per year [4]. Cocoa pod husk constitutes 70 %–75 % of the cocoa pods weight which are often discarded after extraction of the beans [5]. For every tonne of fresh beans, 2 tonnes of fresh pod husk are produced as waste [6]. This can lead to increases in phytosanitary issues since if the untreated CPH was added to the soil serves as reservoir of pests and pathogens that can infect cultivated plants [7]. Cocoa generates about 20 % of Ghana's foreign exchange incomes, making the economy highly dependent on its cultivation, which is largely carried out by small farms that account for around 60 % of the agricultural sector [8]. In Ghana, cocoa waste management is closely linked to national and international

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policies. At the national level, environmental policies and cocoa sector development strategies emphasize sustainable farming practices and the reduction of environmental pollution from agricultural residues [9]. At the international level, improper disposal of organic waste conflicts with global commitments such as the United Nations Sustainable Development Goals (SDGs), particularly SDG 12 (responsible consumption and production) and SDG 13 (climate action), which call for improved waste management and reduced emissions from biomass decomposition. Therefore, developing sustainable strategies to valorise CPH aligns with national and global policy frameworks aimed at promoting environmental protection, climate resilience, and sustainable rural livelihoods.

Strategic solutions for managing waste produced in cocoa plantations, including the valorisation of pod husks ([10,11]; Doungous et al., 2018; [12]), can be achieved through innovative biotechnologies such as vermitechnology. This process relies on earthworms to metabolize and degrade organic waste under aerobic conditions [13], due to the presence in the earthworm gut of microorganisms and enzymes that catalyse the hydrolysis of organic compounds, producing an excrement known as wormcast [14]. This leads to the production of an amendment known as vermicompost, rich in nutrients and active microorganisms that are useful in promoting soil health and plant growth [15]. Because earthworm activity maintains predominantly aerobic conditions, vermicomposting shortens processing time compared with conventional composting. It also minimizes energy consumption, odours, and pathogens, without needing the high temperatures required for biochar production.

Not all earthworms species are suitable for vermicomposting because some main criteria must be fulfilled, including being surface feeders (i.e., epigeic species), being able to adapt to variable conditions such as temperature, humidity, and pH [16,17], and having a high rate of consumption, digestion and transformation of organic matter (OM), to favour the stabilization process [18]. The species commonly used for vermicomposting are *Eisenia fetida* and/or *Eisenia andrei* known for their ability to naturally colonize organic substrates, to tolerate fluctuations in temperature and humidity within moderate stress thresholds, and for its short life cycle [16,19]. However, it has been proven that the efficiency and health of *E. fetida*/*E. andrei* decrease at environmental temperatures higher than 26 °C [20,21], making them vulnerable to the hot climates typical of many African countries [22]. Consequently, these species can be susceptible to environmental stress. The earthworm species *Eudrilus eugeniae* (African nightcrawler) can also be used for vermicomposting. This species is native to tropical West Africa, although it is commonly used in America and European countries as fishing baits. The main advantage of this earthworm is its size (adults can reach 2.5 g in weight), which allows it to consume a considerable volume of food per day [16], making it, under optimal conditions, a prolific feeder. In tropical cocoa production systems, the use of heat-tolerant species such as *E. eugeniae* represents an innovative solution [23]. This species can enhance the efficiency of vermicomposting, enabling scalable and eco-friendly conversion of waste into high-quality soil amendments, supporting sustainable cocoa cultivation.

Although the CPH show promising potential as a biomass for valorisation, the integration of various technologies involving its bioconversion, such as composting, vermicomposting, and pyrolysis, remains largely unexplored. Some studies suggested the combination of CPH-derived compost or vermicompost with other amendments (e.g., biochar), leveraging their synergistic effects to improve soil health and crop productivity [12,24,25]. However, a more effective strategy could involve adding biochar at the beginning of the bioconversion process (e.g., during vermicomposting) of diverse feedstocks (e.g., CPH and manure). This would produce an amendment known as vermichar, rich in enzymes and nutrients derived from earthworm metabolic activity and the adsorption properties of biochar [24]. The importance of adding biochar at the beginning of the vermicomposting process arises from the fact that its porous structure and large surface area is able to adsorb soluble organic molecules, gases, inorganic compounds and contaminants present in the starting matrices or generated during the vermicomposting process, preventing their release into the environment. To the best of our knowledge, this innovative approach has not yet been investigated in the existing literature (i.e., vermichar from CPH).

It is important to gain more knowledge about vermitechnology to valorise agricultural waste such as CPH, which represent a threat for the sustainability of agricultural activities. Furthermore, the use of *E. eugeniae* in vermicomposting represents an original way to exploit this species in vermichar production process. To date, no study has examined the combined addition of biochar at the outset of a vermitechnological process with CPH, using *E. eugeniae* as the decomposer species. This research therefore aims to address this knowledge gap. Accordingly, we hypothesized that exploring the association of *E. eugeniae* and biochar during vermicomposting could valorise CPH and improve both the process and the quality of the final soil amendment. Following this approach, a mixture of fresh CPH and cattle manure was amended with CPH biochar and subsequently vermicomposted by *E. eugeniae* earthworms. The following issues were evaluated during the vermicomposting process: i) the chemical properties, microbial counts, and composition of the different matrices during the vermicomposting process; ii) the dynamics of bacterial communities during vermicomposting; and iii) the maturity and safety of the final amendments.

2. Materials and methods

2.1. Experimental set up

Fresh CPH, obtained from cocoa farms in Assin-Fosu, Ghana (5°40′37″N 1°17′42″W), were mixed with cattle manure (1:1 ratio), then CPH biochar (B) was added at 0 % (control), 4 % and 8 % w/w (0 %B, 4 %B, and 8 %B respectively, Fig. 1). The biochar rates were chosen after a preliminary toxicity test performed in triplicate over a 28-day period (data not shown). Mixtures of cattle manure and CPH were mixed with increasing doses of CPH biochar from 0 to 24 % by weight (0 %B, 2 %B, 4 %B, 8 %B, 16 %B and 24 %B). The 8 % biochar was the highest dose at which no earthworm mortality was observed.

The biochar was obtained through a slow low-temperature pyrolysis (350–400 °C) of previously crushed and dried CPH [12]. The chemical features, microbial count and composition of the feedstock used were reported in Table S1. The pH range of the starting materials was between 6.7 and 7.4 (Table S1). Cocoa pod husk showed the highest concentration of C (i.e. 41 %), whereas cow manure presented the highest concentration of N (1.65 %), with C/N ratios ranging from 17.8 to 33.4 (Table S1). As expected, the cattle manure had a higher concentration of microorganisms, both bacteria and fungi than the cocoa pod husk (Table S1).

The organic raw materials (24.5 kg) were mixed, placed in plastic bins and pre-treated for a period of 30 days before adding the earthworms (Fig. 1). The bottoms and sides of the bins were perforated (0.5 cm diameter) to facilitate the drainage of the water inside, at the same time preventing the earthworms from escaping, while bins lids were perforated (2.0 cm diameter) to facilitate the aeration of the substrate. The feedstock materials were manually mixed and wetted to 60–70 % moisture [13]; they were then carefully turned and homogenized twice a week during the pre-processing phase (lasting 30 days) to allow adequate aeration of the biomass. This was carried out to ensure that pH, C/N ratio, and temperatures reached acceptable values for earthworms [26,27]. During the pre-treatment phase, temperatures peaked at 51 °C and dropped below 30 °C by the end. The pH remained stable around neutrality (i.e., pH ∼ 6.7–7.0), while the C/N ratio decreased from initial values of 23–28 to 16–20 at the end of pre-treatment. Furthermore, the pre-processing phase ensured an initial fragmentation of raw material and degradation of organic compounds, thus reducing the presence of substances toxic for earthworms [28].

After the 30-day period, earthworms (*E. eugeniae*; African nightcrawler) were manually spread on the surface of each container and the

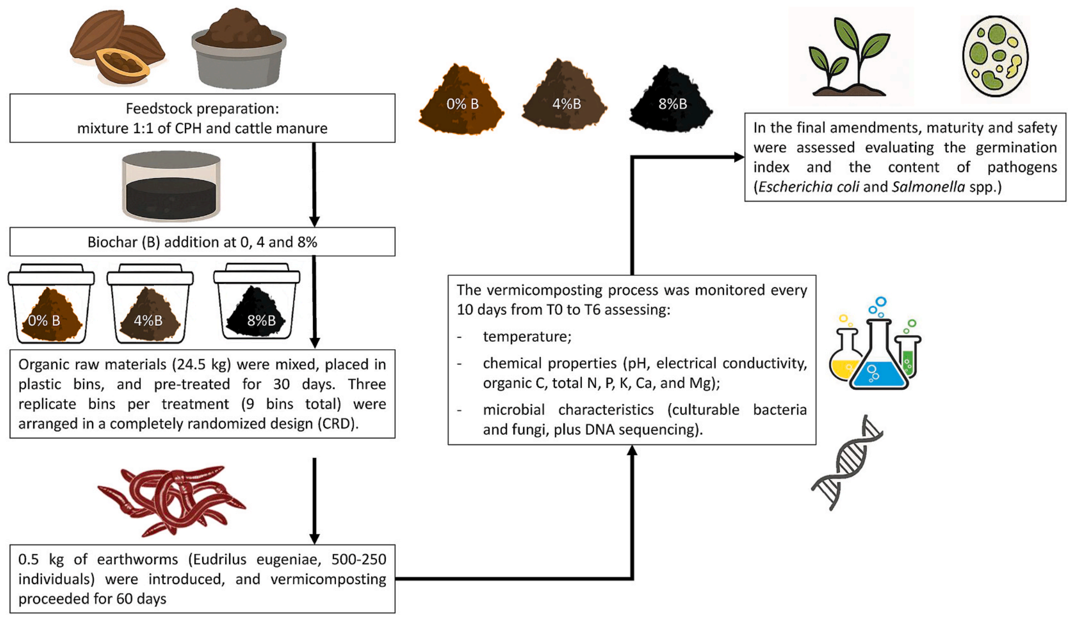

Fig. 1. Experimental design.

mixtures were vermicomposted for 60 days. African nightcrawlers were provided by Green Cycle Technology, Accra, Ghana.

Each plastic bin contained 24.5 kg of the mixture raw materials and 0.5 kg of earthworms (500-250 individuals) (Table 1). Three replicate bins were maintained for each treatment (9 containers in total) arranged in a completely randomised design (CRD).

Throughout the experimental period, temperature was measured and recorded at least twice a week using a digital thermometer. For each container, three measurements were taken at both lateral sides and at the centre. The recorded temperatures during the vermicomposting period are reported in Fig. S1. At the end of the experiment, no earthworm mortality was observed. During vermicomposting, both the earthworm population and total biomass increased, ultimately reaching approximately twice the initial biomass (from 0.5 kg to ~1.0 kg). This increase could be attributed to the growth of the adults introduced at the beginning of the experiment (first generation) and to the birth of new individuals (second generation); as the life cycle of *Eisenia eugeniae* is approximately 60 days, allowing the worms to complete their life cycle during the vermicomposting process [23]. Consequently, the mixture of CPH and cattle manure, with or without CPH-derived biochar, did not exhibit toxic effects on the earthworms.

2.2. Chemical analysis during vermicomposting process

Immediately before adding the earthworms (T0), and then every 10 days (T1, T2, T3, T4, T5, and T6), representative samples (100 g) were randomly collected from all containers, both from the surface (0–5 cm) and from the bottom (15–30 cm). A total of 7 samples were taken during the process. From the fresh samples collected, earthworms were removed, then the samples were oven-dried at 40 °C for 48 h, ground and sieved to 2 mm for chemical analyses, which were performed in triplicate for each trial. The pH and electrical conductivity (EC) were determined in a 1:2.5 water-substrate suspension. Total organic carbon (TOC) was determined by the Walkley-Black wet oxidation method [29]. Total nitrogen (N) was quantified by the Kjeldahl digestion and distillation method according to Okalebo et al. [30]. To determine total P, Ca, Mg and K, samples were prepared and digested according to Hunter et al. [31] and Jones and Case [32]. The content of total P was analyzed according to Bray and Kurtz [33]. Ca and Mg were quantified by titration with EDTA and Calgon red indicator solution, and K by flame spectrophotometry method according to Logah et al. [34]. In the final sample (T6), inorganic N ($N\text{-}NO_3^-$ and $N\text{-}NH_4^+$) was extracted in a 2 M KCl solution (1:10 w: v) and quantified by the Kjeldahl method (ISO 14256–2:2005).

Table 1
Treatments set up for vermicomposting process.

Treatment	Earthworm (kg)	Cow Manure (kg)	Cocoa Pod Husk (kg)	Biochar (kg)
0 %B	0.5	12	12	0
4 %B	0.5	11.52	11.52	0.96
8 %B	0.5	11.04	11.04	1.92

2.3. Microbiological analyses

During the vermicomposting period, fresh samples (from which the earthworms were removed) were taken every 10 days (T0, T1, T2, T3, T4, T5, and T6) to quantify the microbial population dynamics, i.e. total culturable bacteria and fungi, and the presence of pathogens (*Salmonella* spp. and *E. coli*, data shown only for T6). All microbial counts were performed in triplicate for each treatment.

For bacterial plate counts, the standard protocol of Cappuccino and Sherman [35] was followed. A 10 g of each sample was added to 90 mL of sterile distilled water and the serial dilution method was used [36]. The abundance of the microbial populations was reported as Log10 colony-forming units per gram of substrate (Log CFU g^{-1}).

For fungal quantification, sabouraud dextrose agar substrate was used and incubated at 37 °C for 24 h as described by Munshi et al. [37].

The concentration of *E. coli* was assessed according to Kornacki and Johnson [38] protocol. A 10 g sample was transferred into 90 mL of buffered peptone water. After appropriate dilutions, the sample was inoculated into a sterile Petri dish and the prepared culture medium (tempered to 45 °C) was poured over it. The samples were then incubated upside down at 45 °C for 18 h. After incubation, the plate colonies were smear-swabbed onto EMB agar plates for identification of typical *E. coli* colonies.

The concentration of *Salmonella* spp. was assessed following the guidelines of the International Organization for Standardization (ISO 6579–1:2017). A 25 g sample was dissolved in 225 mL of buffered peptone water and incubated at 35 °C for 20 h. Then, 0.1 mL of the pre-enriched sample was pipetted into 10 mL of Rappaport-Vassiliadis soya peptone broth (RVS broth) and incubated at 42 °C for 48 h. Thereafter, a full loop of the RVS broth was streaked on Xylose Lysine Desoxycholate Agar (XLD agarand incubated at 35 °C for 24 h for colony identification.

2.4. DNA extraction and high-throughput sequencing of bacterial communities

Total genomic DNA was extracted in quadruplicate for each bin at T0, T3 and T6 (250 mg, 4 replicates from sample), using the PowerSoil® DNA isolation commercial kit (MoBio Laboratories, Inc., California, USA), following the manufacturers' protocols. After isolation, the DNA concentration and purity were performed using a NanoDrop One (Thermo Fisher Scientific), measuring the absorption coefficients A260/A280 and A260/A230. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified by PCR using the 515F (5′-GTGCCAGCMGCCGCGGTAAT-3′) and 806R (5′-GGACTACHVGGGTWTCTAA-3′) primer pair, and sequenced using the Illumina Miseq platform. Sequencing was performed at Novogene (Beijing, China) commercial facility. Raw sequences obtained from Illumina sequencing were initially processed using QIIME2 (v.2020.2). DADA2 version 1.10 was used to denoise the reads, correct errors and generate Amplicon Sequence Variants (ASV). Taxonomic assignment for ASVs was carried out with the RDP Classifier (Version 2.2).

2.5. Phytotoxicity bioassay test

Phytotoxicity tests were conducted in triplicate on sub-samples of the final vermicompost/vermichar using *Zea mays* (corn) seeds according to the procedure described by Zucconi et al. [39]. Corn was selected as an indicator crop due to its importance for Ghanaian agriculture, accounting for over 50 % of the country's cereal production. It also serves as a representative model for other plants that could be grown on the soil amendments produced. Moreover, corn is one of the standard recommended species in the Organisation for Economic Co-operation and Development (OECD) terrestrial plant toxicity test.

A 1:10 (w/v) mixture of vermicompost/vermichar and distilled water was prepared, shaken at 150 rpm for 1 h, centrifuged at 4000 rpm for 20 min and then filtered. Dilutions of 30 % (30 % extract + 70 % distilled water), 50 % (50 % extract + 50 % distilled water) and control (distilled water only) were prepared from each sample. A Petri dish filled with sterilised filter paper was wetted with 5 mL of extract. Ten seeds of corn were placed on the filter paper, and the Petri dishes were incubated randomly at a temperature of 25 ± 2 °C in the dark. After 3 days of incubation, germination percentage and root length were recorded. The germination index (GI) was calculated as follows (1):

$$GI\ (\%) = [(Gt\ \%)\ /\ (Gc\ \%)] \times [Lt\ /\ Lc] \times 100 \quad (1)$$

where:

Gt % and Gc % = the mean germination percentage of germinated seedlings on the extract and control respectively.

Lt and Lc = the mean root lengths of the seedlings grown in the extract and control respectively.

2.6. Economic feasibility analysis

Tröltzsch et al. [40] applied a simplified cost-benefit analysis to evaluate the economic and practical feasibility of the vermitechnology for small-scale farmers. The method calculated the net profit and the benefit-cost ratio (BCR) for the production of a batch of vermicompost/vermichar. This involved quantifying all direct and indirect costs related to the production process, including the initial outlay for labour and materials, as well as ongoing operating costs. The following key economic parameters were considered:

Total Cost (TC): sum of all variable and fixed costs for producing a batch of amendment.

Variable Costs (VC): vary with the production levels and include raw materials (fresh CPH, cattle manure, earthworms, and CPH biochar) and labor costs (estimated based on the average hourly wage of Ghanaian workers) for activities such as collecting CPH, mixing, watering, turning, and harvesting.

Fixed Costs (FC): one-time or infrequent costs spread over the production lifecycle, including plastic bins and tools (per batch, based on estimated lifespan) and land costs.

The Total Cost (TC) is calculated as follow (2):

$$TC = VC + FC \quad (2)$$

Total Benefit (TB): total revenue generated from the sale of the final amendment. It can also be defined as the cost-savings derived from replacing comparable commercial organic fertilizers. To calculate the benefit, the total mass of the marketable product (vermicompost/vermichar) produced per batch and the current market price of comparable quality organic fertilizers in Ghana are used.

The Total Benefit (TB) is calculated as follow (3):

$$TB = \text{Mass of Final Amendment (kg)} \times \text{Market Price per kg (GH₵)} \quad (3)$$

Net Profit (NP): the difference between the total benefits and the total costs is calculated as follow (4):

$$NP = TB - TC \quad (4)$$

Benefit-Cost Ratio (BCR): a key indicator of profitability. A BCR greater than 1 indicates that the benefits outweigh the costs and is calculated as follow (5):

$$BCR = \frac{TB}{TC} \quad (5)$$

2.7. Statistical analysis

All chemical and microbial analyses were performed in triplicate on the samples collected from each container and the mean values ± standard errors are shown in the tables and figures. All statistical analyses of repeated measurements were performed in R [41]. The dataset was analyzed to assess the effects of different treatments on multiple response variables over time, with time treated as a repeated measure. Using the packages = "*nlme*" and "*emmeans*", generalized linear mixed models were applied to evaluate the effects of treatment, time, and their interactions. Replicate was included as a random effect to account for variability over time within the three replicates.

Statistical analyses of the final amendments features were carried out using the NCSS 2007 Data Analysis software (v. 07.1.21; Kaysville, Utah).

One-way analysis of variance (One-way ANOVA) was carried out to compare mean values of each parameter of the final amendments. When significant *p*-values ($p < 0.05$) were obtained, the differences between individual means were compared using the Fisher's post-hoc least significant difference test (LSD, $p < 0.05$).

Alpha diversity metrics (Caho1, Shannon) were calculated to assess the richness and evenness of the bacterial communities within each sample. To compare the bacterial community composition between different samples, beta diversity was analyzed using Bray-Curtis distance metric. Non-metric Multidimensional Scaling (NMDS) was used to visualize the beta diversity patterns. Differentially abundant taxa across treatments were identified with the "analysis of compositions of microbiomes with bias correction (ANCOM-DB), and linear discriminant analysis effect size" The pH values found in the final (LEfSe) methods.

3. Results and discussion

3.1. *Dynamics of the chemical parameters during the vermicomposting process*

3.1.1. *Dynamic of pH and electrical conductivity*

Over the vermicomposting period the pH of the treatments ranged from 6.84 to 8.72 (Fig. 2A) and its variation in the substrates was affected by the time and the treatment ($p < 0.0001$; Table S2). All treatments showed the lowest pH at the beginning of the process (T0); over time, the pH increased and reached a peak between T1 and T2 (Fig. 2A). With the addition of the earthworms, aerobic conditions were enhanced (due to the burrowing activities of the earthworms), which could have favoured an increase in the pH of the biomass, as a probable consequence of the more intense degradation of carboxylic acids, or their condensation/polymerization into more complex molecules [42]. At the end of the vermicomposting process, the 8 %B treatment reached a pH value of 8.72, ~1.10 times higher than that of the 0 %B and 4 %B treatments. This can be ascribed to the high pH value of the biochar compared to that of the other feedstocks (Table S1). In addition, the

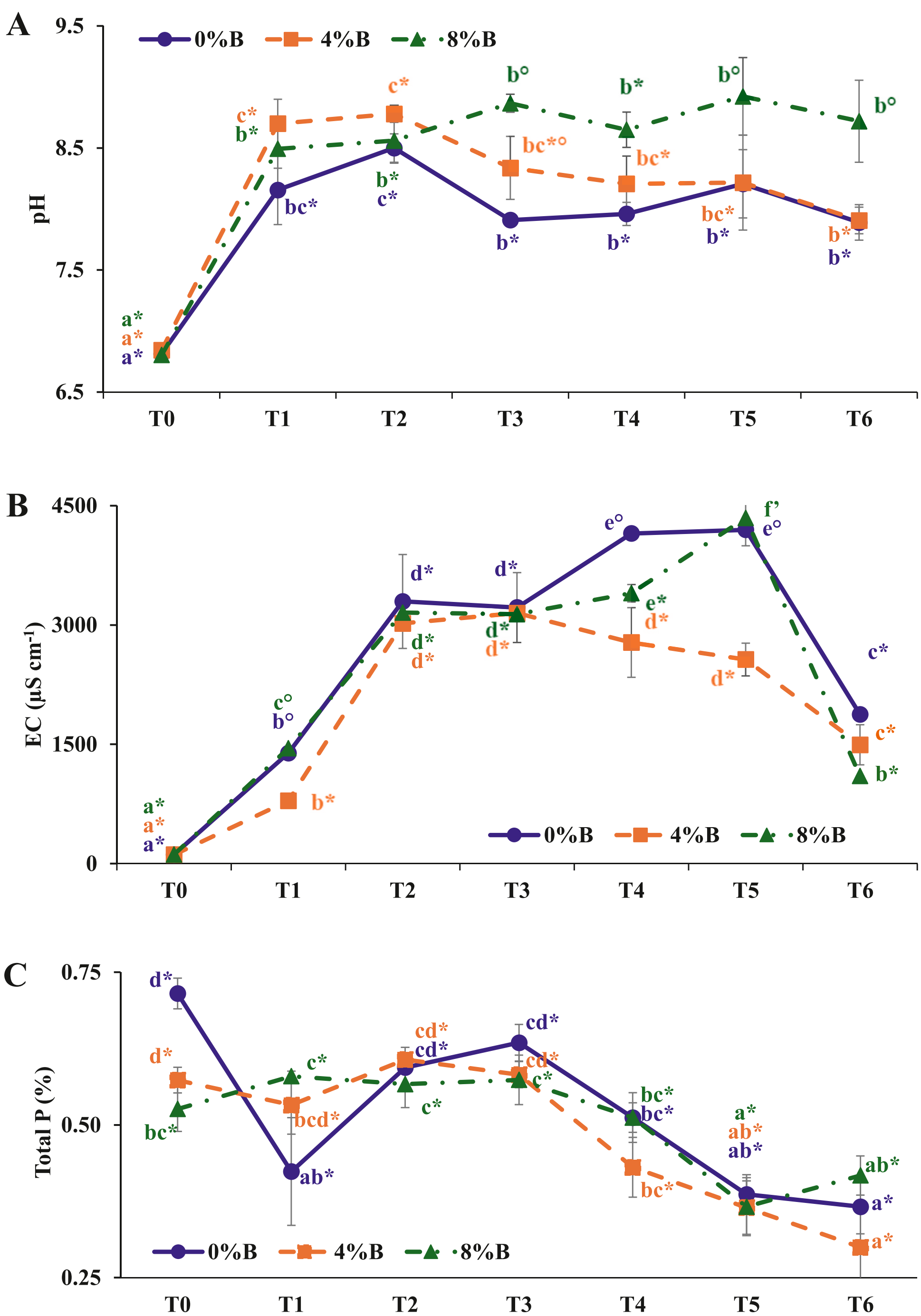

Fig. 2. Dynamics of chemical parameters during the vermicomposting process: (A) pH; (B) Electrical conductivity (EC); (C) Total P. Mean values ± SE (n = 3) within the same treatment (0 %B, 4 %B and 8 %B) followed by different letters of the same colour indicate statistically significant differences. Different symbols (*,°,') for each time indicate statistically significant differences between the three treatments (0 %B, 4 %B and 8 %B). Both letters and symbols are based on the generalized linear mixed model and its post-hoc comparisons ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

adsorption capacity of biochar towards low molecular weight acids released during the decomposition of OM was also responsible for the increase in pH of vermichar at 8 %. The pH values found in the final materials are in line with those of vermicompost obtained from different feedstocks (e.g. pomace, sewage sludge, peat straw, cow and pig manure) treated with biochar [43–47], and are suitable values for the use of these materials as soil amendments Regulation [48].

The increase in EC during the vermicomposting period was affected by time, biochar application ($p < 0.0001$) and their interaction ($p = 0.0007$; Table S2). EC reached a first peak at T2 and, after a stable period, it increased presenting a second peak at T4 (for 0 %B) and T5 (for 8 %B; Fig. 2B), while for 4 %B, the EC remained stable between T2 and T5. Subsequently, all treatments showed a decrease in EC in T6 (Fig. 2B). The reduction in EC observed at the end of the

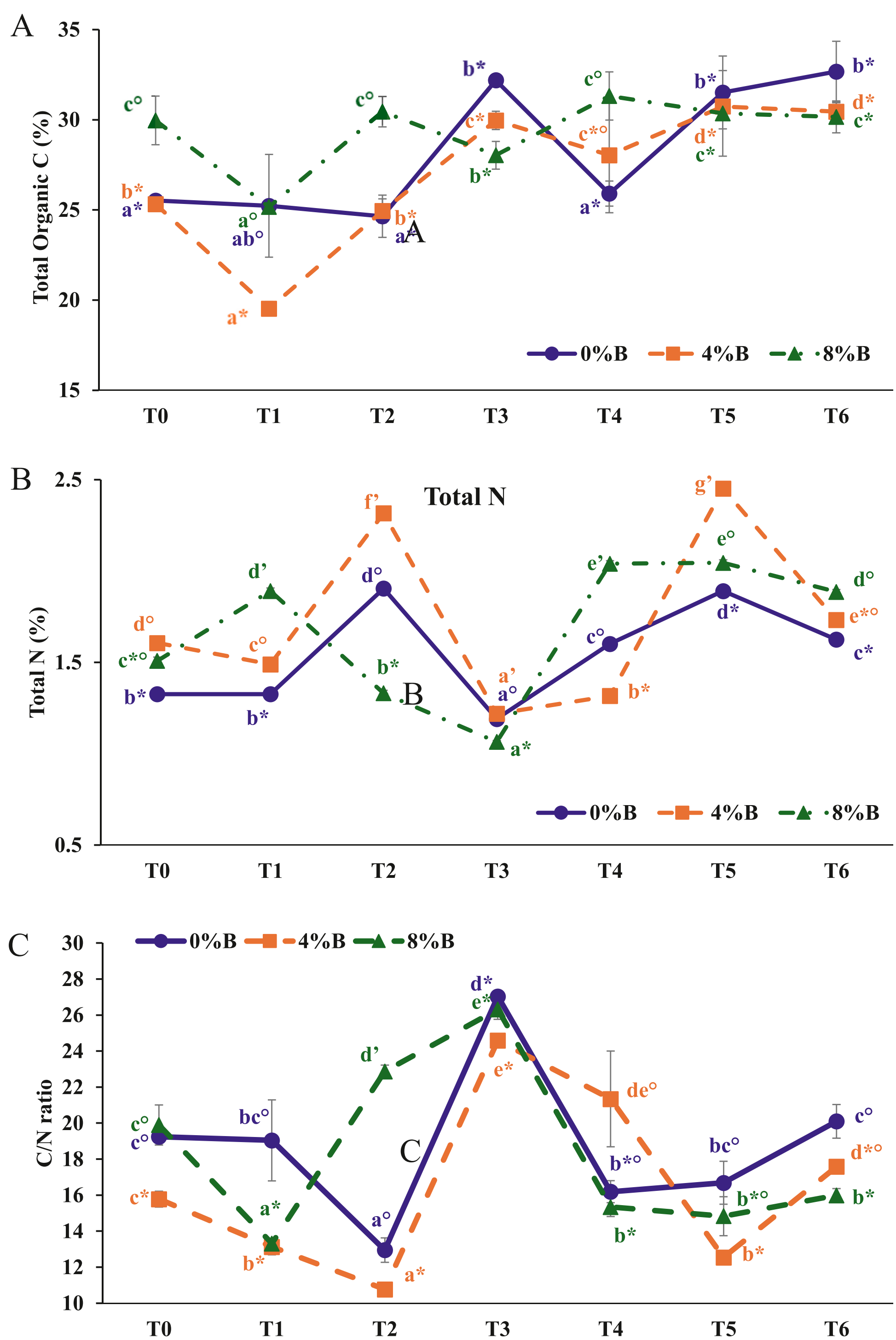

Fig. 3. Dynamics of chemical parameters during the vermicomposting process: (A) Total Organic C (TOC); (B) Total N; (C) C/N ratio. Mean values ± SE (n = 3) within the same treatment (0 %B, 4 %B and 8 %B) followed by different letters of the same colour indicate statistically significant differences. Different symbols (*,°,') for each time indicate statistically significant differences between the three treatments (0 %B, 4 %B and 8 %B). Both letters and symbols are based on the generalized linear mixed model and its post-hoc comparisons ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

vermicomposting period (i.e. T6) can be due to a reduction in the concentration of soluble ions caused by leaching or their precipitation as insoluble salts; these decreases could also be due to the ingestion of ions by the earthworms [44,49]. After 60 days of vermicomposting the EC was the highest in the 0 %B treatment (1875 $\mu S\ cm^{-1}$), which was higher than the EC of the 4 %B (1493.33 $\mu S\ cm^{-1}$) and 8 %B (1100 $\mu S\ cm^{-1}$) treatments. The addition of biochar probably resulted in a higher retention of soluble ions on its surface, leading to lower EC. These results are in line with those reported by Li et al. [47], who tested the vermicomposting of cow manure grain straw treated (or not) with biochar. The vermicompost and vermichar have EC values at the end of the process that allow them to be used as soil amendments, as EC values > 5000 $\mu S\ cm^{-1}$ are detrimental to soil and plant growth [50].

3.1.2. Dynamic of phosphorus content

A general trend of decreasing total P concentration during the vermicomposting period was observed in all treatments (Fig. 2C). Only time ($p < 0.0001$) and time*treatment ($p = 0.0396$) influenced P concentration, as the addition of biochar did not significantly affect this parameter (Table S2). Between T0 and the end of the vermicomposting period (T6), the total P concentration decreased 1.95-, 1.91- and 1.26-fold in the 0 %B, 4 %B and 8 %B treatments, respectively. These decreases could be attributed to phosphorus ingestion by earthworms and its utilisation by the associated microbiota, which convert organic P into inorganic forms that can be more readily assimilated by earthworms [24]. Since earthworms were removed prior to analysis, the P incorporated into their bodies did not contribute to the measured total P pool in the vermicompost/vermichar samples. Finally, losses due to leaching of inorganic phosphate released during organic matter degradation must be considered. These results agree with those reported by Ghosh et al. [51], who observed a decrease in organic phosphorus due to the utilisation of P by earthworms during the vermicomposting process of various organic wastes. In any case, the P content in the final amendments was below the minimum content required by the European guidelines for organic amendments (0.44 % P per gram of amendment, Regulation [48].

3.1.3. Dynamic of carbon and nitrogen content

TOC was affected by time ($p < 0.0001$), treatment ($p = 0.0096$) and their interaction (p-value = 0.0053; Table S2). In all treatments, a slight increase in TOC content over the vermicomposting period, was observed (Fig. 3A); it is assumed that the greatest losses of C in the form of CO_2 occurred during the pre-processing phase (30 days), and that the subsequent vermicomposting was only a curing period regarding the carbon dynamics within the substrates. Biochar is a C-rich material [52], so its addition, especially at 8 %, increased the C concentration of the substrate (e.g. +1.2 times in the 8 % treatment compared to 0 %B in the period T0 to T4; Fig. 3A). Earthworm-driven biotransformation during vermicomposting may have indirectly contributed to TOC enrichment. The passage of organic compounds through the earthworm gut may have stimulated microbial activity and protected the more recalcitrant carbon fractions, which accumulated in the castings [53]. These findings align with those reported by Lin et al. [54] and Nigussie et al. [55], who reported that earthworms stabilize and protect organic carbon during vermicomposting through its fragmentation and mucus addition. The apparent increase in TOC observed during vermicomposting can also be attributed to dry mass loss, which concentrated the recalcitrant carbon fraction (e.g. Ref. [56]). The final amendments showed a TOC content between 30 and 33 %, which is in line with that of vermicomposts obtained from different organic materials (e.g. spent beer grains, pig or cow manure, crabgrass straw, grape bagasse) in combination (or not) with biochar [19,43,57,58]. TOC values in vermichar (compost) are in line with European guidelines Regulation [48] which classify as organic fertilisers those materials with a TOC concentration above 15 %.

The N content was significantly affected by time, biochar treatment and their interaction ($p < 0.0001$; Table S2). The dynamics of the total N content of vermicompost and vermichar did not show a clear trend, and the addition of CPH biochar affected the N content differently (Fig. 3B). The fluctuation of N content during vermicomposting can be due to the alternating immobilisation and release of N by earthworms and associated microorganisms [59]. Total N in the final amendments increased as the percentage of biochar increased (+~5-fold for 4 %B and 8 %B, compared to the 0 %B treatment). It is known that biochar is able to adsorb organic compounds and ions [52], thus reducing the rate of OM mineralisation and limiting N losses through volatilization (i.e. N-NH_3) or leaching (i.e. N-NO_3^-). Therefore, the higher the content of biochar in the starting materials, the lower the loss of N [45,58,60]. The total N content in CPH amendments is in line with that of organic fertilisers, and it is higher than the minimum N content proposed by the European regulation (EU regulation 2019/1009).

The content of N-(NO_3^-) at the end of the process (i.e. T6) was 6.28- and 5.47-fold higher in 4 %B and 8 %B vermichar treatments, compared to the vermicompost (0 %B), while N-(NH_4^+) showed an inverse trend, decreasing with increasing biochar rate (<1.6 and 1.2-fold for 4 %B and 8 %B, respectively, compared to 0 %B; Table 2). The higher quantity of oxidized forms of N [N-(NO_3^-)] in the biochar-containing treatments suggests that the high porosity of biochar improved aerobic conditions, which, in combination with the microorganisms associated with the earthworm gut, may have stimulated the nitrification process [61]. The ratio N-(NH_4^+)/N-(NO_3^-) <0.5 at the final time (0.21 and 0.02 for vermicompost and vermichars respectively) highlights that the 60 days of process produced stable amendments [62].

The C/N ratio was affected by time ($p < 0.0001$), treatment ($p = 0.0002$) and their interaction ($p < 0.0001$; Table S2). For all treatments, the C/N ratio reached its maximum value at T3 (Fig. 3C). Thereafter, a reduction was observed at T4 until reaching final values of 20, 18 and 16 for 0 %B, 4 %B, 8 %B respectively at T6. A C/N ratio below 20 is an index of the stability reached by the vermicompost [47]. Therefore, in all of the final amendments obtained (0 %B, 4 %B, 8 %B), the final C/N ratio indicated an advanced transformation process of OM. This ensured the production of a stable final amendment, i.e. with reduced biological activity resulting from the decomposition of most of the degradable organic matter reaching optimal C/N ratio values, that can be safely used in agriculture [63,64].

3.1.4. Dynamic of calcium, magnesium and potassium content

The Ca^{2+} content in all the amendments decreased over the vermicomposting period (Fig. 4A), and it was affected by time ($p < 0.0001$), treatment ($p = 0.0002$) and their interaction ($p < 0.0001$; Table S2). The addition of biochar did not change Ca^{2+} concentration at the end of the process (T6). Regarding Mg^{2+} content, an overall decrease affected only by the time ($p < 0.0001$; Table S2) was observed during the vermicomposting period (Fig. 4B). The observed reductions in Ca^{2+} and Mg^{2+} could be due to the ingestion and accumulation of these ions by earthworms and/or their leaching during the vermicomposting process. The content of K^+ was affected by time ($p < 0.0001$), treatment ($p = 0.0023$) and their interaction ($p = 0.0004$; Table S2). The 0 %B and 4 %B showed a similar decreasing trend over, compared to 8 %B treatment which had a higher K^+ concentration in the final compost after 60 days than the

Table 2
Properties of mature vermicompost (0 %B) and vermichar (4 %B and 8 %B).

	0 %B	4 %B	8 %B
N-(NH_4^+) ($g\ kg^{-1}$)	0.06 ± 0.00^c	0.04 ± 0.00^b	0.03 ± 0.00^a
N-(NO_3^-) ($g\ kg^{-1}$)	0.29 ± 0.00^a	1.82 ± 0.03^c	1.58 ± 0.02^b
Salmonella spp.	absent	absent	absent
Escherichia coli (CFU g^{-1})	520 ± 1.13^b	680 ± 1.21^c	10 ± 0.71^a
30 % extractant GI (%)	48.23 ± 8.78^a	107.59 ± 9.93^b	142.81 ± 1.34^c
50 % extractant GI (%)	109.97 ± 8.44^b	86.89 ± 13.82^{ab}	52.99 ± 20.22^a

Mean values ± SE (n = 3) followed by different letters within a row denote statistically significant differences between treatments (0 %B, 4 %B and 8 %B), according to the Fisher's Least Significant Difference (LSD) test ($p < 0.05$).

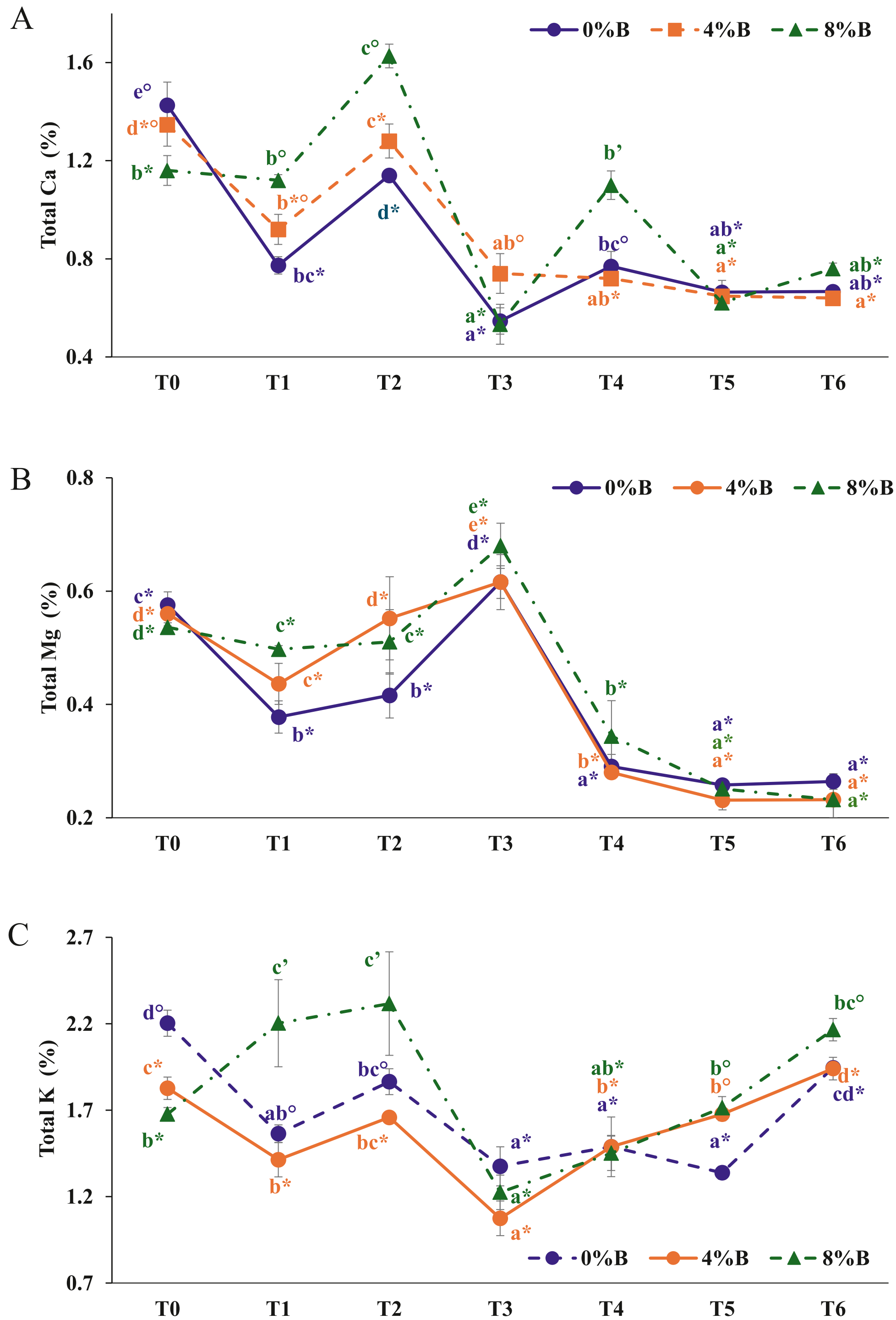

Fig. 4. Dynamics of chemical parameters during the vermicomposting process: (A) Total Ca; (B) Total Mg; (C) Total K. Mean values ± SE (n = 3) within the same treatment (0 %B, 4 %B and 8 %B) followed by different letters of the same colour indicate statistically significant differences. Different symbols (*,°,') for each time indicate statistically significant differences between the three treatments (0 %B, 4 %B and 8 %B). Both letters and symbols are based on the generalized linear mixed model and its post-hoc comparisons ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

starting materials at T0 (Fig. 4C). At the end of the vermicomposting process, the highest K^+ concentration was found in the 8 %B treatment (e.g. +1.28 times in T6 compared to the control; Fig. 4C). This is attributable to the biochar's ability to specifically adsorb K^+ ions, thereby regulating their utilisation by microorganisms and earthworms and reducing K^+ leaching, as also reported by Soobhany et al. [65]. The K^+ content in the final amendments makes them suitable for agricultural use as organic fertilisers, as they meet the requirements of European legislation regarding the concentration of this essential element Regulation [48].

Taken together these results highlight that the use of biochar during vermicomposting process had a positive effect on the final properties of the amendments, with an increase in pH and in the concentration of certain nutrients (N and K) and a reduction in the C/N ratio in the 8 %B.

Moreover, *E. eugeniae* has proven to be suitable for the bio-valorisation of CPH, being versatile in the digestion of different organic materials such as cocoa pod husks. Furthermore, since it is a species native to the study area (i.e. Ghana), its use in the vermicomposting process can help maintain ecological integrity, promote native species and preserve local fauna from invasive exotic species, in accordance with the Sustainable Development Goals (SDG 15.5 and 15.8) of the United Nations AGENDA 2030, as well as facilitate its adoption by local farmers.

3.2. Microbial dynamics over the vermicomposting process

3.2.1. Evolution of culturable bacteria

Over the vermicomposting process time ($p < 0.0001$), treatment ($p = 0.0264$) and their interaction ($p < 0.0001$; Table S2) affected the bacterial population. The addition of earthworms to the substrates resulted in an initial decrease (between T0 and T1) of bacterial counts in all treatments (Fig. 5A). From T4 time, the counts remained stable until T6, presenting at the end of the process values 1.69 and 1.70 times higher in treatments 4 %B and 8 %B, respectively, compared to the control (0 %B) (Fig. 5A). Similar trends, characterized by an initial decline in bacterial counts followed by a stabilization phase, have been reported in other vermicomposting studies. These dynamics are generally attributed to the competition between earthworms and microbes for C and N, along with the earthworms' intensive grazing and gut transit, which selectively digest part of the microbial communities. This process decreases certain bacterial populations while promoting the growth of others that are better adapted to the gut environment [43,49,59,66,67]. The subsequent stabilization of microbial counts likely reflected the establishment of a new dynamic equilibrium between earthworm grazing and bacterial regrowth, supported by the formation of new microhabitats, such as earthworm casts, biochar pores and newly formed organic aggregates. Similarly, Wu et al. [68] found that 5 % biochar addition significantly enhanced bacterial biomass during cattle dung vermicomposting. Moreover, Lv et al. [69] reported increased bacterial diversity during vermicomposting compared to conventional composting, particularly when biochar and similar amendments were included.

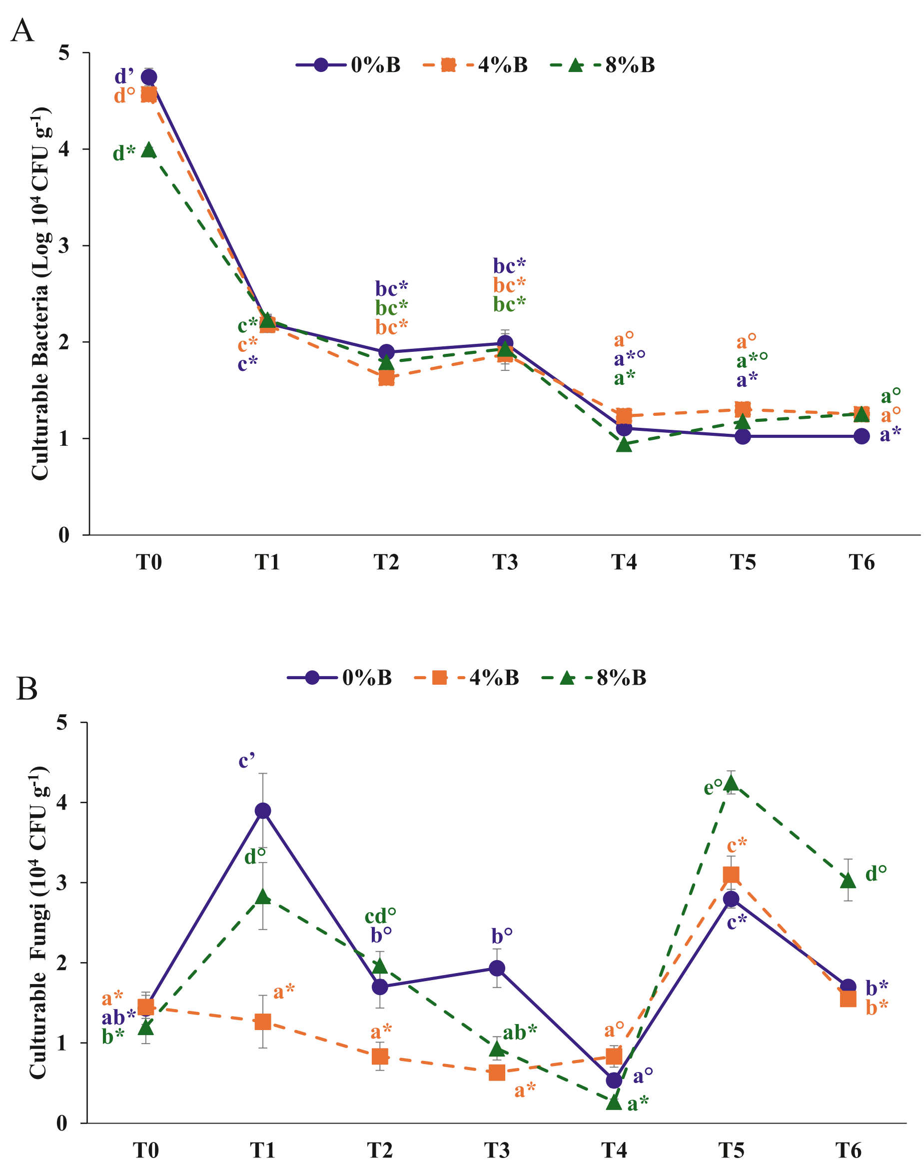

Fig. 5. Dynamics of microbial parameters during the vermicomposting process: (A) Culturable Bacteria; (B) Culturable Fungi. Mean values ± SE (n = 3) within the same treatment (0 %B, 4 %B and 8 %B) followed by different letters of the same colour indicate statistically significant differences. Different symbols (*,°,') for each time indicate statistically significant differences between the three treatments (0 %B, 4 %B and 8 %B). Both letters and symbols are based on the generalized linear mixed model and its post-hoc comparisons ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2.2. Evolution of culturable fungi

The fungal population was affected by time, biochar application and their interaction ($p < 0.0001$; Table S2). The number of fungi showed a variable trend in all treatments, fluctuating between growth and decline phases, with significant increases at T1 (except for the 4 % B treatment) and T5. At the end of vermicomposting (T6), the 8 %B treatment showed a higher fungal abundance than the other treatments (1.96 and 1.78 times higher than the 0 %B and 4 %B, respectively; Fig. 5B); highlighting a long-term stimulating effect of biochar when added at high concentrations (i.e. 8 %).

At the end of the process, the treatment with the highest dose of biochar showed a greater abundance of cultivable bacteria and fungi per unit mass of amendment, suggesting that changes in the chemical properties of vermichar 8 %B (particularly the increase in the content of total P, K and N) rendered it more effective in supporting the metabolic needs of microorganisms. Furthermore, the large specific surface area and porosity of the biochar may have promoted the growth of microorganisms, as the pores of the biochar can be a suitable habitat for microbial colonisation [70,71]. The variable fungal population trends, with biochar treatments showing a higher abundance at T6, are consistent with Antunes et al. [72] and Awasthi et al. [73], which reported the suppression of fungi during the thermophilic phase, followed by a recovery only during the mesophilic maturation phase.

3.3. Microbial community diversity analysis during vermicomposting of cocoa pod husks

In the samples without biochar (0 %B), both the Chao1 and Shannon indexes decreased by day 30 (T3), indicating a reduction in bacterial ASVs richness as well as diversity and evenness (Table 3). The recovery of both indexes by day 60 (T6) suggested that the bacterial communities were reorganized after an initial period of instability. Samples 4 %B and 8 %B showed a significant increase in both the Chao1 and Shannon indexes from T0 to T6, suggesting a progressive enhancement in species richness and a more uniform distribution of bacterial ASV. These results can be related to the biochar's porous structure, which protected the microorganisms from environmental stress and enhanced their activity [71]. Additionally, as previously observed, the biochar influenced the availability and retention of nutrients, thereby supporting the development of diverse microbial communities [60].

Ordination analysis confirmed that biochar addition significantly influenced the microbial community assembly during vermicomposting (Fig. S2). The most marked differences were observed between the 0 %B and 8 %B samples (ANOSIM R = 0.95, $p < 0.01$). Compared to the 0 %B sample, the 4 %B and 8 %B samples showed greater variations across the three timepoints analyzed (T0, T3, and T6). These differences were smaller at days 30 and 60 than at day 0, suggesting that biochar addition to the raw materials resulted in a converging microbial community composition during vermicomposting.

Table 3
Alpha diversity indexes of microbial communities. 0 %B: control treatment (cocoa pod husks: cattle manure at 1:1 ratio); 4 %B: control + 4 % biochar; 8 %B: control + 8 % biochar. T0: before vermicomposting; T3: 30 days after vermicomposting; T6: 60 days after vermicomposting.

Sample	Timepoints	Chao1	Shannon
0 %B	T0	323.8 ± 32.22 [ab]	4.91 ± 0.09 [ab]
	T3	300.5 ± 19.40[b]	4.73 ± 0.11[b]
	T6	327.0 ± 9.42[a]	5.01 ± 0.07[a]
4 %B	T0	305.3 ± 2.31[b]	4.76 ± 0.17[b]
	T3	322.8 ± 10.31 [ab]	4.97 ± 0.05 [ab]
	T6	342.0 ± 5.20[a]	5.05 ± 0.19[a]
8 %B	T0	319.7 ± 8.08[b]	4.82 ± 0.08[b]
	T3	321.0 ± 14.79 [ab]	4.96 ± 0.07 [ab]
	T6	331.3 ± 9.32[a]	5.01 ± 0.03[a]

[a-d] Different letters in the same column represent significant differences, as determined by ANOVA followed by Tukey-HSD test (adjusted p value = 0.01).

3.4. Taxonomic distribution of microbial communities

3.4.1. Shifts in dominant phyla and genera

Actinomycetota, Pseudomonadota, Bacillota, and Bacteroidota were the dominant phyla in all the samples analyzed, in accordance with previous studies on vermicomposting [74,75] (Fig. S3). Actinomycetota and Pseudomonadota are commonly found among the dominant phyla in vermicompost [76];. they are crucial for soil fertility and plant growth due to their roles in OM degradation, N fixation, biocontrol activities, and increased nutrient availability. The relative abundance of Bacillota increased significantly in the control samples (0 %B) from T0 to T6, being highly represented in the biochar-amended substrates (4 %B and 8 %B) since T0. This is consistent with previous observations that the abundance of Bacillota increases in earthworm cast following the addition of 2 % and 6 % biochar to the rearing substrates [73]. Additionally, Bacillota grows during the passage through the earthworms' gut, particularly the aerobic and potentially denitrifying *Bacilli* [77]. On the contrary, the abundance of Bacteroidota decreased during the vermicomposting period, reaching the lowest values in biochar-amended substrates. In this respect, the observed increase in vermichar pH from 6.0 to 8.7 may have negatively affected the abundance of Bacteroidota, as these bacteria typically prefer neutral environments [78]. Of particular interest is the phylum Nitrososphaerota, consisting of ammonia-oxidizing archaea (AOA), which showed an abundance 3 to 10 times higher in the 8 %B samples compared to the other treatments. During composting and vermicomposting, Nitrososphaerota can convert ammonium (NH_4^+) to nitrite (NO_2^-), which can then oxidize into nitrate (NO_3^-). In substrates with a pH above 8.0, such as those here amended with 8 % biochar, Nitrososphaerota can reduce N loss *via* ammonia volatilization, thus increasing the N concentration in the final material. Consistently, the 8 %B samples exhibited the highest concentration of nitrate ($N{-}NO_3^-$) and the lowest ammonium N ($N{-}NH_4^+$) levels at the end of the process. This pattern suggests that the enrichment of Nitrososphaerota combined with the high pH of this treatment were promoted nitrification and favoured N retention. Additionally, the concurrent increase in the relative abundance of Bacillota and decrease in Bacteroidota could be related to the alkalinization observed in the 8 % B vermichar.

To better evaluate the microbial communities during vermicomposting in response to biochar addition, taxonomic composition was also assessed at the genus level (Fig. 6). Considering all the samples and timepoints analyzed, the most represented genera (>4 % relative abundances) were *Streptomyces, Pelagibacterium, Rhabdothermincola, Lysinibacillus, Micromonospora and Mesorhizobium*. *Streptomyces* (Actinomycetota) is frequently isolated from vermicompost and plays important roles in soil, such as the biological control of plant pathogens, and the solubilization of phosphate [79]. Similarly, *Micromonospora* (Actinomycetota) is a plant growth-promoting bacterial genus, able to solubilize phosphate. *Mesorhizobium* (Pseudomonadota) was already identified as one of the dominant N-fixing bacteria in vermicompost obtained from sludge, cow dung, and vegetable wastes [80]. *Pelagibacterium* (Pseudomonadota), *Lysinibacillus* (Bacillota) and *Rhabdothermincola* (Actinomycetota) were already isolated from compost and vermicompost, they have demonstrated a remarkable adaptability to extreme conditions [81] and are involved in the decomposition of OM in the soil [82,83]. The lowest C/N ratio value at T6 in the 8 %B sample can be attributed to the increased abundance of N-fixing bacteria and the nitrogen retention mechanisms provided by the porous structure of biochar. Moreover, the presence of phosphate-solubilizing genera *Streptomyces* and *Micromonospora* might produce a possible P availability enhancement, that could be beneficial for agricultural application.

3.4.2. Identification of biomarker taxa

To identify biomarker taxa, LEfSe analysis was carried out (Fig. 7).

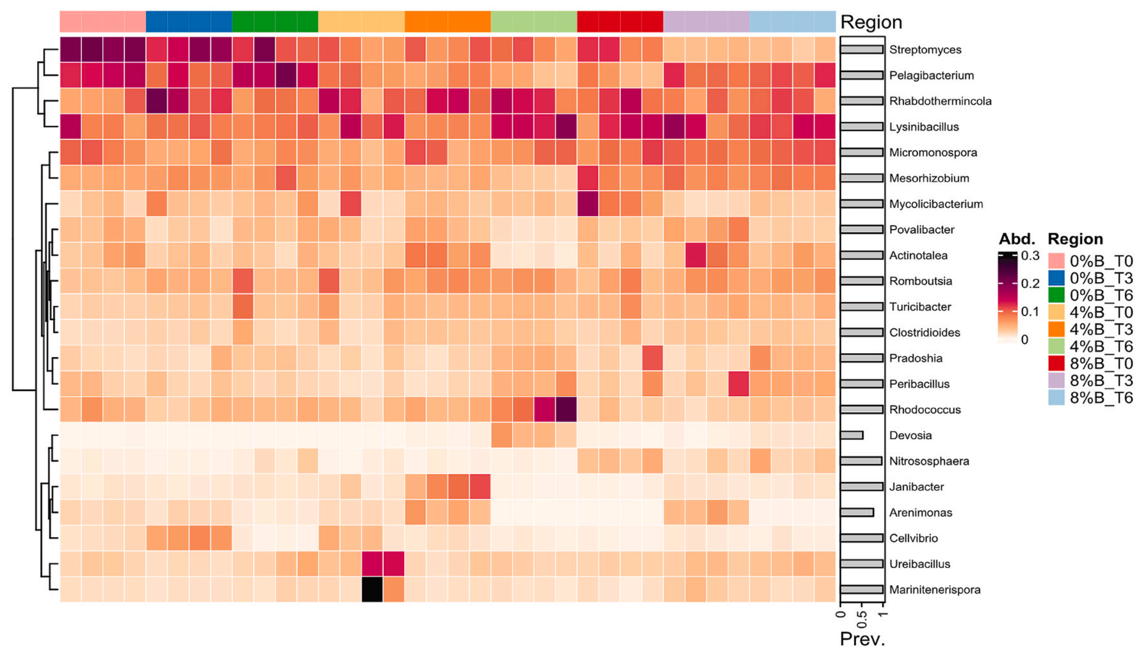

Fig. 6. Relative abundance of the dominant bacterial and archaeal genera during vermicomposting of cocoa pod husks. The top 20 taxa were selected based on the frequency of occurrence of ASVs classified to each genus across all samples. The distribution histogram of relative abundance of species and clustering tree based on the Weighted Unifrac were reported in the graph. 0 %B: control treatment (cocoa pod husk: cattle manure at 1:1 ratio); 4 %B: control + 4 % biochar; 8 %B: control + 8 % biochar.

Streptomyces and *Pelagibacterium* were significantly associated with the control samples (0 %B) at all the time points analyzed. Other genera differentiating the 0 %B samples were *Rhabdotherminicola*, *Cellvibrio* and *Cytobacillus* at T3, as well as *Nitratireductor, Sorangium,* and *Thermocrispum* at T6 (Fig. 7). *Cellvibrio* (Pseudomonadota) is known for its cellulolytic activity [84]. Characterization of amylases produced by *Cytobacillus* (Bacillota), suggests that this genus could have a role in OM decomposition in moderately acidic to neutral soils. On day 60, the presence of *Nitratireductor* (Pseudomonadota) suggests an active N cycle in the 0 %B. *Sorangium* (Myxococcota) is a cellulolytic myxobacterium known to produce compounds with biocontrol potential. *Thermocrispum* (Actinomycetota) contributes to the breakdown of recalcitrant OM, enriching the vermicompost with stable organic compounds beneficial for soil structure. Taken together, the biomarker genera detected in the 0 %B vermicompost suggests the development of a microbial consortium with a strong potential to improve soil organic matter stability and suppress plant pathogens, rather than enriching the substrate with nutrients. The 4 %B samples were characterized at T0 by *Arenimonas*, *Cellvibrio* and *Ureibacillus*. *Arenimonas* (Pseudomonadota), which comprises soil species often associated with the degradation of organic compounds. *Ureibacillus* (Bacillota) has important roles in compost and waste decomposition and has been proposed for biotechnology applications [85]. A marked shift occurred in 4 %B samples at T6, with *Rhodococcus*, *Lysinibacillus*, *Peribacillus*, *Devosia*, and *Cytobacillus* differentiating these samples. *Rhodococcus* (Actinomycetota) is known for its capacity to degrade a wide range of organic pollutants, promoting the decomposition of complex carbon sources. *Lysinibacillus* and *Peribacillus* (Bacillota) have plant growth-promotion and biocontrol properties [86]. Finally, the presence of *Devosia* (Pseudomonadota), a N-fixing genus, suggests an increasing potential for N enrichment in the 4 %B samples. The co-occurrence of involved in organic pollutant degradation and plant-beneficial taxa in the 4 %B vermichar suggests that this amendment can enhance soil fertility and health. Specifically, *Rhodococcus*, due to its ability to break down a wide range of recalcitrant and potentially harmful organic compounds, may contribute in the 4 %B to the restoration of degraded or moderately contaminated soils. Meanwhile, *Lysinibacillus* and *Peribacillus* can promote plant growth and biocontrol, and *Devosia* contributes to N fixation, indicating that the 4 %B vermichar can also aid in recovering soil fertility.

During the vermicomposting process *Mesorhizobium* and *Nitrososphera* (Nitrososphaerota) were the biomarker taxa of vermicompost samples amended with 8 % biochar. As previously stated, these genera are critical for N fixation and ammonia oxidation and are thus central to N cycling. Accordingly, Malińska et al. [87] found that biochar addition could not only promote OM stabilization, but also increase N content by reducing ammonia volatilization. Other genera associated with 8 %B sample at T0 and T3 were *Micromonospora* and *Ureibacillus* (Bacillota), which are microbial genera with plant growth-promoting and OM decomposition potential. In 8 %B, the simultaneous enrichment of *Mesorhizobium, Nitrososphera, Micromonospora* and *Ureibacillus* is consistent with the chemical characteristics of this treatment, which showed the highest total N and N-(NO_3^-) concentrations, and the lowest N-(NH_4^+)/N-(NO_3^-) ratio. Overall, 8 %B vermichar appears as a nutrient-rich biostimulant amendment with a strong capacity to retain N in its oxidized forms.

Overall, the analysis of the dynamics of bacterial communities during vermicomposting indicated that, although significant differences were identified at the genus level, the three treatments resulted in the enrichment of microbial taxa associated with OM degradation, biocontrol, and N cycling processes. The identified correspondence between microbial and chemical dynamics suggests that the three final amendments differ not only in their microbial composition but also in their functional potential. However, the impact of the microbial communities of the amendments on soil and plants should be investigated in further studies.

3.5. Maturity and safety of the vermicompost and vermichar

The maturity of the final material (vermicompost and vermichar), i. e. the extent to which the amendment has fully undergone its transformation process and is deemed safe for use in agricultural production, was assessed by quantifying *E. coli* and *Salmonella* spp. and determining

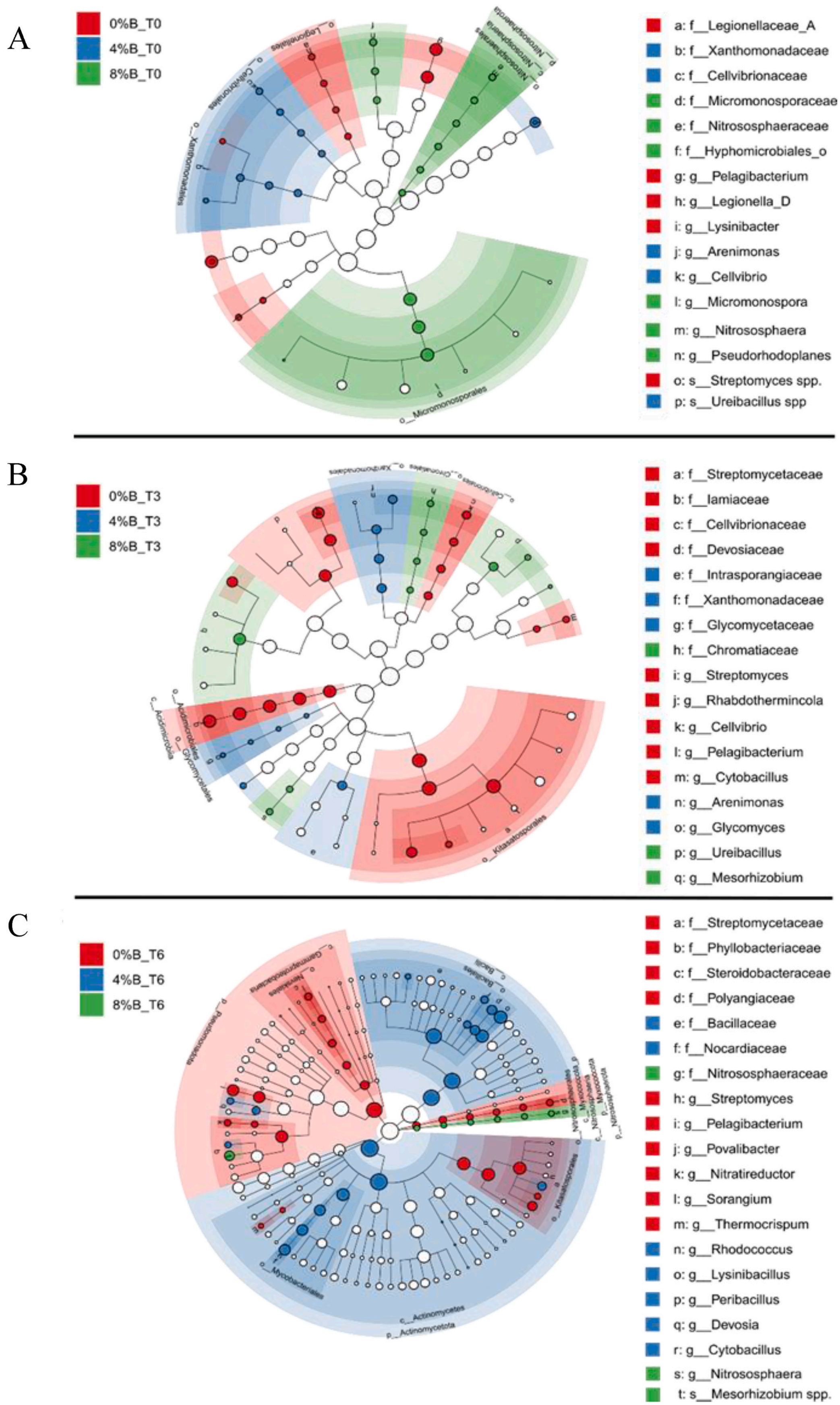

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Fig. 7. Cladogram representing microbial taxa discriminative among samples analyzed at time 0 (panel A), after 30 days (Panel B) and after 60 days (Panel C), as determined by LEfSe analysis. (Red) control treatment (cocoa pod husk: cattle manure at 1:1 ratio); (Blue) control + 4 % biochar; (Green) control + 8 % biochar. The size of nodes represents the abundance of taxa. Only taxa meetings a LDA significant threshold >2, with a *p* value < 0.05, are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the germination index by means of a germination test.

Salmonella spp. was not detected in any of the treatments (Table 2), while all materials showed low *E. coli* counts (between 10 and 680 CFU g^{-1}), below the permitted EU threshold for organic amendments (between 1000 and 5000 CFU per g of amendment; [48]. The 4 %B treatment showed the highest *E. coli* count (680 CFU g^{-1}), followed by the 0 %B treatment (520 CFU g^{-1}) and the 8 %B treatment, where the lowest *E. coli* number (10 CFU g^{-1}) was observed (Table 2). These results show that vermicomposting of CPH residues was able to produce mature soil amendments (vermicompost and vermichar) useful for a safe agricultural development.

The germination index (GI) was evaluated on the final products diluted to 30 % and 50 % with distilled water. In the 30 % dilution, the GI increased with the application of biochar, with 8 %B showing the highest GI (142.8 %), which was 1.62- and 1.33-fold higher than the 0 % B (88.2 %) and 4 %B (107.6 %) treatments (Table 2). The ability of biochar to affect the chemical and microbiological characteristics of the final material favoured maize germination at the at higher dilutions. The 50 % diluted biochar treatments showed lower GI than the control (GI = 86. 9 %, 53.0 %, and 110.0 % for 4 %B, 8 %B and C, respectively). It should be considered that a high GI (above 80–100 %) suggests that the amendment is mature and safe for plants, while a GI below 50 % may signal the presence of toxic substances. The negative influence on the GI observed in the samples receiving higher biochar doses with limited dilution (i.e. 50 %) can be attributed to the possible presence of phytotoxic substances and persistent free radicals that generate reactive oxygen species [88,89]. This could have induced oxidative stress, lipid peroxidation and cell membrane damage [88], ultimately impairing seed enzyme activity and root elongation. Moreover, biochar at 8 % increased soil pH and cation content (particularly K^+) further imposing additional ionic and osmotic stress. This aligns with findings of Carril et al. [89], who reported that liquid biochar extracts and solid biochar showed higher toxicity than washed biochar, owing to the leaching of soluble toxic substances. The GI also indicated that, at the highest dilution for vermichar (30 %) and the lowest for vermicompost (50 %), the amendments can exert a biostimulant activity on plants. Conversely, the 8 % treatment with low dilution resulted in a GI very close to the threshold value of 50 % for potential toxicity. This underscores the need for a thorough pre-assessment of the phytotoxic effects of biochar related to the amounts added and the dilutions performed.

3.6. Economic feasibility analysis

The vermicomposting process from CHP and cattle manure (with biochar addition) required only modest cash inputs, as most materials were low- or no-cost local by-products. (Table S3). The FC were mainly for containers and basic equipment. The TC for amendment production ranged from 41.55 to 56.55 GH₵ (3.21–4.36 €) per batch, varying with purchased inputs such as cattle manure, biochar, and earthworms (Table S3). Based on the substitution value of commercial organic fertilizers, the calculated benefits amounted to approximately GH₵ 60.00 (€ 4.63) per batch, with an NP ranging between GH₵ 3.45–18.45 (€ 0.27–1.42) and a net return of GH₵ 0.17–0.92 per kg of amendment produced (Table S4). The benefit–cost ratio of 1.06–1.44, confirms that small-scale production of cocoa pod husk–based vermicompost/vermichar is economically attractive under the tested conditions (Table S4). These BCR values align with those reported by Devkota et al. [90] and confirmed vermicomposting's profitability. Compared to the profitability of cocoa production (e.g. GH₵ 1.8 per kg of cocoa beans and net profits exceeding GH₵ 600 ha^{-1} net profits; [91]), CHP vermicomposting serves best as an additional, rather than alternative, strategy to enhance income and soil fertility.

Furthermore, to ensure the scalability of the tested process, it is essential to address several key priorities. These include a more detailed assessment of economic feasibility under real agricultural conditions, considering the biochar costs, labour requirements, and potential financial returns from higher yields or reduced fertilizer use. Developing low-cost reactor systems, such as modular beds, stackable trays or semi-passive windrows, will be essential for smallholders with limited resources or fluctuating residue volumes. Additionally, standardized guidelines are needed for managing earthworm feed rates, moisture regulation, biochar dosing, and earthworm maintenance in tropical environments.

4. Conclusions

The valorisation of the CPH through the vermicomposting with *E. eugeniae* resulted in mature, stable, and nutrient-rich organic amendments suitable for soil application. Since *E. eugeniae* is native to West Africa, and it can be also recovered in the wild near cocoa farms, its use supports ecological integrity (by avoiding the introduction of non-native species) and fosters local adoption, rendering it particularly suitable for smallholder farms. The addition of biochar at the beginning of the process improved the chemical characteristics of the final product. Microbiologically, it enhanced bacterial and fungal diversity and abundance. The amendments were also pathogen-free: all final treatments showed complete absence of *Salmonella spp.*, *E. coli* levels well below EU regulatory limits, and no presence of phytosanitary risks.

The vermicomposting system tested produced a valuable amendment that reduces the need for chemical pesticides and helps protect both crop and soil microbiomes. Additionally, the nutrient-rich vermichar can partially replace synthetic fertilisers, reducing the upstream emissions. From an environmental perspective, the process can provide quantifiable benefits since CPH is often left to decompose anaerobically or burned, generating methane or CO_2 emissions. Redirecting CPH into an aerobic vermicomposting system substantially can reduce greenhouse gas emissions associated with uncontrolled decay.

To enable broad implementation, future research should address scalability challenges. Additionally, long-term field trials are needed to evaluate the impacts of CPH vermichar on soil health, carbon persistence and crop productivity across different seasons and management practices. Addressing these aspects will strengthen the feasibility and dependability of CPH vermicomposting systems for sustainable cocoa production.

CRediT authorship contribution statement

Benjamin Amedi Afful: Writing – original draft, Investigation, Formal analysis. **Patrick Enchill:** Writing – review & editing, Data curation. **Michael Osei:** Writing – review & editing, Investigation. **Samuel Twum Adjei:** Writing – review & editing, Data curation. **Laura Atuah:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Vincent Logah:** Writing – review & editing, Supervisor, Methodology. **Mauro Lo Cascio:** Writing – review & editing, Formal analysis. **Angela Bianco:** Writing – review & editing, Methodology. **Giacomo Zara:** Writing – review & editing, Formal analysis. **Marilena Budroni:** Writing – review & editing, Validation, Conceptualization. **Tom Sizmur:** Writing – review & editing, Methodology, Conceptualization. **Matteo Garau:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Paola**

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Declaration of 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2026.108988.

Data availability

Data will be made available on request.

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