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Cultivating Biodiversity to Harvest Sustainability: Vermicomposting and Inoculation of Microorganisms for Soil Preservation and Resilience

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Abstract: Based on the concepts of circular economy and bioeconomy, the reuse of agrifood residues through vermicomposting can help solve serious environmental problems such as soil contamination and degradation, erosion and climate change. In this sense, the objective was to identify, quantify and analyze the physical, chemical, hormonal, amino acid content and microbial biodiversity of three formulations of vermicompost, with and without inoculation of microorganisms from native forest and commercial formulation, aiming at the production of an organic fertilizer rich in microorganisms for use in sustainable production systems. As a result, the vermicompost formulations presented values higher than the minimum requirements stipulated by Brazilian legislation for the registration of class A composite organic fertilizer. There is a significant difference between the vermicomposts, in the parameters related to the content of phosphorus, auxin, tryptophan and organic matter, as well as the relation between humic and fulvic acids. *Bacillus* sp. and *Trichoderma* sp. were also influenced by the type of vermicompost formulation. In addition, inoculation with microorganisms from native forest promoted an increase in biodiversity, in which the presence of *Actinomyces* sp. and *Azotobacter chrooccocum* contribute to the reduction in the levels of heavy metals in the compost. It is concluded that vermicomposting is a potential tool in the reuse of agri-food residues, with expressive microbial diversity that can influence plant growth, suppression of pathogens, minimize or reduce the effects of biotic and abiotic stresses on plant production, in addition to contributing to maintenance of soil biodiversity, integral fertility and resilience to climate change.

Keywords: social technology; bioeconomy; beneficial microorganisms; humic substances; climate change; sustainable production

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1. Introduction

The world is expected to demand 70% more food by 2050, which represents a major challenge in terms of sustainability and agricultural pressure on natural resources. In accordance with the United Nations Food Organization's Sustainable Development Goals (SDG) and the guidelines of the Intergovernmental Technical Panel on soils, in the face of the challenging scenario of climate change, degradation, loss and imbalance of resources and environmental systems, researchers a world level argue that sustainable land management is critical to achieving SDGs, food security and climate change mitigation [1].

Globally, large amounts of solid wastes are produced daily. Among these, organic waste from urban and agricultural centers are the major components of solid waste [2]. The inadequate disposal of these residues favors the proliferation of pests and disease vectors, in addition to generating contaminants such as heavy metals, greenhouse gases,

eutrophication of water resources and soil degradation [3], going against the grain of sustainable development goals (SDGs).

According to Zago and Barros [4] the World Economic Forum has pointed to the circular economy and the bioeconomy as a model that allows the re-signification of the concept of "waste" to "resource", as it can be reintroduced in the food production chain in order to reduce the impact of the sector on natural resources and reduction of production costs. Based on this paradigm shift, the "zero waste" program aims by 2030 to increase the reuse of waste by 70%, as the use of organic waste can help solve serious environmental problems such as soil degradation, erosion, and climate change.

In Brazil, animal and vegetable residues are underutilized. Of all organic waste, 37 million tons reach the processing units, but only 1% of this is directed to composting units in the country. Vermicomposting and biodigestion as recycling tools are not widely used, wasting residues that can be reinserted into agricultural systems in the form of organic fertilizers enriched with efficient microorganisms [5].

Among the recycling techniques, vermicomposting is a non-thermophilic biological transformation process in which the organic material is converted into an organic fertilizer with high porosity, water retention capacity, and high microbial activity through interactions between earthworms and associated microorganisms in the digestive tract and in the mass to be decomposed [6].

The available literature reports that the inoculation of efficient microorganisms combined with vermicomposting can reduce decomposition time, provide an increase in nutrients such as total nitrogen and phosphorus, increase the content of humic substances and stabilization of the fraction organic, in addition increases the carbon content, which helps in the microbial structuring of the soil [7,8]. It can also increase soil biodiversity, act in the production and release of phytohormones that impact on plant metabolism [9], in the suppression of pathogens in organic residues and soil phytopathogens [2–9].

Furthermore, vermicomposting is considered a low-cost technology, where the transformation of the residue into fertilizer and its subsequent application to the soil can reduce and/or replace the dependence on conventional fertilizers, provide nutrients, and improve the soil structure [10–12].

In this sense, "planting biodiversity to harvest sustainability," in order to preserve and improve life below ground through technological tools in organic waste management based on the bioeconomy and zero waste, are especially important for a global increase in food production in challenging conditions such as drought or low soil fertility, especially in the tropics [6,13].

Research focused on understanding the microbial diversity of organic fertilizers such as vermicompost will be prerequisites in the future to better understand the influence on development, primary and secondary metabolism of plants, as well as the plant-soil-microbiome interaction, in recovery and restoration of degraded soils for the advancement of sustainable agrifood systems, food production and food sovereignty [6,14].

The objective of this study was to analyze, identify, and quantify the mineral elements, content of humic substances, phytohormones, amino acids, and microbial communities of three formulations of vermicompost, with and without inoculation of efficient microorganisms from native forest and commercial formula, in order to produce an organic fertilizer rich in microorganisms for application in agricultural systems.

2. Materials and Methods

2.1. Inoculum Preparation and Activation: From Native Forest and Commercial Formulation

Approximately 700 g of unsalted rice was cooked in distilled water (DW). After cooking, the rice was placed on plastic trays with perforations at the bottom to prevent water from accumulating, and covered with a thin screen to protect the contents from animals or weather. The rice trays were distributed on native forest soil around the Federal University of Lavras, where they remained for a period of 15 days. The microbiological traps were spaced within a radius of 20 m from each other, and the local litter was placed

on the tray, aiming to also collect part of the local cellulolytic microbiota Coutinho, within the premises of the Federal University of Lavras-UFLA. The litter covering the soil was placed on the tray, in order to take advantage of the natural microbiota of the environment.

After this period, the microorganisms present in the pink, bluish, yellowish, and orange capture medium were used for the activation process, in which they were placed in a 20 L container, followed by homogenization with 1 L of sugarcane juice and distilled water until a total volume of 20 L. For 20 days the container was stored in a closed, cool and ventilated place until it presented an orange color with a pleasant sweet smell. The preparation of the inoculum from native forest was conducted according to the Notebook of Efficient Microorganisms [15].

Commercial inoculum (Korin®) was prepared according to the manufacturer's instructions. It consisted of a solution obtained by diluting the concentrated inoculum in water and cane molasses in the following proportions: 10% molasses (sugarcane), 80% water, and 10% concentrated commercial inoculum. Part of the water was placed in the 20 L container, the molasses was added and after homogenization, the concentrated inoculum was added, followed by water to complete the volume. The gallon with the inoculum was stored in a place protected from the sun for a week.

Both commercial and soilborne inoculum were inoculated during the experiment setup process, at time zero (0), homogeneously with the raw material in the corresponding masonry niches 5 L of the respective inoculum were added with 15 L of water, totaling 20 L in each cell.

2.2. Vermicompost Formulation

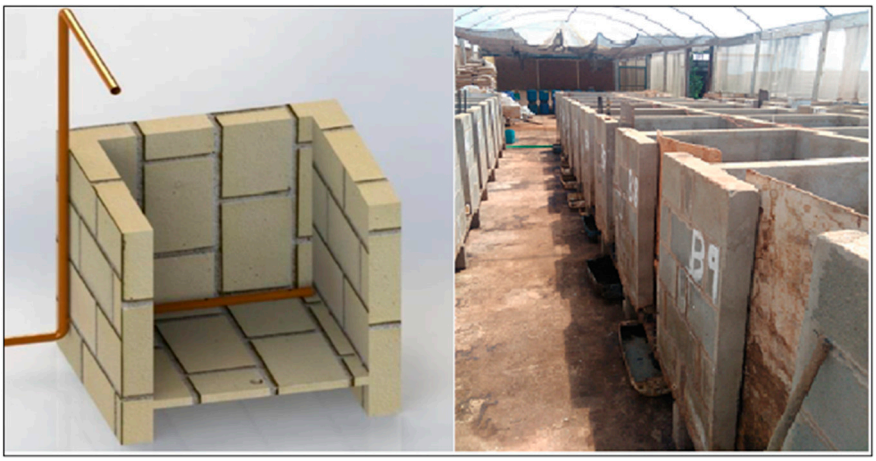
The vermicompost was produced in the Biodiesel Sector of UFLA in two stages: composting to stabilize the material followed by vermicomposting. The raw material used consisted of food waste from the University Restaurant-RU, among leftovers from lunch and meal preparation, such as rice, prepared beans, pineapple skins, lettuce, beetroot, carrots, cabbage, and chard. Lignocellulosic residue from pruning and landscape management within the university was also used to correct the C/N ratio.

The composting process was applied to food waste from the university restaurant and plant waste from campus landscaping (pruning and plant management) without prior treatment, conducted in masonry blocks with a volume of 1 m^3 (each cell) (Table 1).

Table 1. Initial characterization of waste.

Feedstock	Carbon	Nitrogen	C/N Ratio
Food Waste	39	0.6	65:1
Landscaping Waste	35.15	2.93	12:1

In the composting phase, three treatments and three replications were conducted, totaling nine experimental units (cells) in completely randomized blocks. The first treatment was conducted without inoculation of microorganisms (V1). The second (V2) was performed with inoculation of soilborne microorganisms (SM) and the third (V3) was inoculated with commercial microorganisms (CM) (Korin®) prepared according to the manufacturer's recommendations. Both treatments were inoculated to a population of 1×10^{-6} CFU $\times$ mL^{-1}, on the first day of the initial composting phase. After 60 days, with the material still in the composting phase, at the beginning of the maturation phase, 20 kg of bovine manure and 0.5 kg of California red earthworm (*Eisenia fetida*) were added to each cell to start the vermicomposting phase, remaining for another 60 days in masonry blocks for biotransformation and full maturation of the vermicompost. The entire material transformation process, from the initial phase to the full maturation of the vermicompost, was conducted in masonry niches (Figure 1) for 120 days.



(**a**) (**b**)

Figure 1. Graphic representation (**a**) and real image (**b**) of masonry structures used for vermicomposting in this study. Reference: Passos [16].

During an average period of 120 days, temperature monitoring was performed (digital thermometer PT10 model MPT2, Lexitron-Guemisa, Madrid, Spain). After the biotransformation of the materials, ten single samples were collected from each cell, which was then homogenized into a single composite sample and sent in triplicate to the Plantsphere Laboratory in Ecuador, to perform the analysis of humic substances, amino acids, microbial content, and parameters such as biological, chemical, and hormonal. The vermicomposts at the time of sample collection, without sieving, showed the following characteristics: bio-oxidation, stable temperature, dark brown color, and humified odor.

2.3. Physical, Chemical, Hormonal, Amino Acid, and Microbial Analysis

The analyzes to quantify and identify the physical, chemical, hormonal, amino acid, and microbial content of the three vermicompost formulations were performed according to the analysis methodology.

2.3.1. Physicochemical Analysis

The physical and chemical analysis were carried out according to the methodologies proposed by [17] in which the organic matter content (% m.s.) was determined by the EN 13039 Standard (2001), the total carbon (% m.s.), by the equation % Total C = % Organic matter × 0.5 About 50 g of vermicompost samples were used, in triplicate, to determine the concentration of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Fe, Cl, Cu, Mn, Zn, B, and Mo) with N concentration determined by the Kjeldahl method, phosphorus by colorimetry, and Ca, Mg, Cu, Fe, Zn and Mn by atomic absorption spectrophotometry. The determination of potassium was performed by flame photometry, whereas the analyzes of copper (Cu), cadmium (Cd), and lead (Pb) concentrations were also performed in the Atomic Absorption Spectrophotometer with flame.

2.3.2. Fraction, Quantification, and Purification of Humic Substances

The chemical fractionation of organic matter into humic acid (HA) and fulvic acid (FA) was conducted based on solubility in acidic and basic media [18] For this, 1 g of matured vermicompost was added with 30 mL of HCl 0.5 mol L^{-1}, followed by stirring for 2 h. The supernatant with the non-humic fraction (SNH) was separated by centrifugation (15 min at 2500 rpm), and the total volume was then measured. To the solid residue, 30 mL of 0.5 mol L^{-1} NaOH was added, stirred for three hours, and centrifuged (15 min at 2500 rpm), repeating this procedure until the supernatant became clear.

In each extraction, the supernatant with soluble humic substances (SH = HA + FA) was transferred to a collecting flask, and the final volume was adjusted to 200 mL. After

removing an aliquot of 20 mL for carbon analysis, the remainder was acidified with HCl 0.5 mol L^{-1} to pH 2.0. After 24 h of rest, the HA, insoluble in acidic pH, was separated by centrifugation from the soluble PA. The C content in the SNH, AF, and SH extracts was quantified spectroscopically, measuring the absorbance at 580 nm, after reaction with an acid solution of potassium dichromate 0.625 mol L^{-1}, for 4 h, at 60 °C, from a standard curve of anhydrous D-glucose in the range of 0 to 200 mg L^{-1} of C.

The C present in the form of HA (HAC) was estimated from the difference between the C of the SH extract (HSC) and the C of the FA extract (FAC). The results were calculated by distributing each fraction as a percentage of total organic carbon and the humification indices: Humic acid index (HI = HAC/HSC.100) and humification ratio (HR = HAC/FAC).

2.3.3. Microbiological Analysis: Serial Dilution and Plating

For the microbiological analysis of vermicomposting, six samples of the material were collected, which were homogenized to form a composite sample, from which an aliquot of 1 g of vermicompost was collected. Afterward, the aliquot was transferred to a test tube containing 9 mL of 1% peptone solution, with this dilution being 10^{-1}. In sequence, serial dilutions were performed, ranging from the dilution of 10^{-2} to 10^{-7} under agitation at 120 rotations per minute (rpm) for 45 min. Samples were conserved in 20% glycerol and distilled water for HPLC analyses.

The plating was conducted in triplicate, placing 100 µL of the dilution on the specific culture medium, spreading the inoculum (dilution) on the culture medium with the aid of a sterilized Drigralsky loop.

For bacteria, culture medium NA (Nutrient-Agar) was used, and for fungi, culture medium PDA (potato-dextrose-agar) was used. Inoculated plates were incubated at room temperature for 24 h. Then, the total count of the plates that presented between 30 and 300 colonies was performed.

Subsequently, each morphotype found was isolated through successive subcultures via compound streaks, in new Petri dishes, containing a clean culture medium. The morphological analysis of the colonies included size, days of growth, shape, elevation, border, surface, mucus production, optical detail, color, and presence or absence of a halo in the GYC medium. Subsequently, the identification of organisms was conducted through isolation in selective media and morphological characterization of colonies [19,20].

2.3.4. Identification of Microorganisms

The procedure for identification and quantification of microorganisms followed the protocols of the Plantsphere Laboratory, Ecuador. For the identification and quantification of microorganisms in (Log CFU·g^{-1}), Biolog's MicroLog® Portable Microbial Identification System was used, which offers basic identification capacity for bacteria, fungi and yeasts. The system's software compared samples of vermicompost treatments, in triplicate, to an extensive Biolog database, identifying a wide variety of microorganisms, according to the laboratory protocol.

2.3.5. Phytohormone Analysis

The quantification of phytohormones was performed by UPLC-ESI-MS/MS of indole-3-acetic acid (auxin), zeatin (cytokinin), and gibberellic acid (gibberellin), in Tryptic Soy Broth (TSB). Phytohormone extraction was performed with ethyl acetate, followed by a partition step. After centrifugation, an aliquot of the supernatant was subjected to drying and the residue was resuspended in a mobile phase (75% of 0.1% formic acid and 25% of methanol). The sample was injected into an ultra-performance liquid chromatograph (UPLC) with a Waters column (Acquity UPLC BEH, C18; 17 µm; 2.1 × 50 mm) coupled to mass spectrometry with an electrospray interface. Instrument parameters were capillary (kV) 3.00; Cone (V) 25.00; Extractor (V) 4.00, Source temperature (°C) 120; Desolvation temperature (°C) 300. The MRM mode was selected, with monitoring of two transitions per analyte. The limits of detection (LD) of the instrument for Zeatin and Indoleacetic

Acid were 0.010 μg mL^{-1} and for Gibberellic Acid it was 0.025 μg mL^{-1}. The limits of quantification (LQ) of the instrument obtained for Zeatin, Indoleacetic acid, and Gibberellic acid were 0.050 μg mL^{-1}. The analytical curve was used in the range of 0.05–1.00 μg mL^{-1}.

2.3.6. Amino Acid Analysis

Samples of 200 mg of vermicompost were collected in triplicate and macerated with 6 mL of 80% ethanol (*v*/*v*) and concentrated in a speed vac until the ethanol was eliminated. Sample volumes were adjusted to 2 mL with water and centrifuged at 20,000 g for 10 min. The supernatant was filtered through a 20 μm nitrocellulose membrane and then used for amino acid determination. Aliquots of 20 μL of the filtrate and 60 μL of the OPA-borate solution, used for the derivatization of amino acids at room temperature, were homogenized for 2 min and then analyzed by HPLC. The fluorescence detector was set to 250 nm excitation and 480 nm emission. 20 μL of the solution derivatized with o-phthaldialdehyde was injected. The areas and retention times of each amino acid were evaluated by comparison with standard amino acids at known concentrations of Tryptophan, Cysteine, Thiamine, Riboflavin, Pyridoxine, Folic Acid, and Nicotinic Acid, according to the methodology proposed by Astarita [21].

2.4. In Vivo Experiments

After the formulation of the three vermicomposts, the field experiment was conducted in the experimental area of the Plant Tissue Culture sector at the Federal University of Lavras (UFLA), under the geographic coordinates: Latitude −21.223062 and Longitude −44.972369. The *O. vulgare* L. specimen was deposited at the ESAL/UFLA herbarium, under registration no. 22,156.

The seedlings were obtained from apical cuttings of mother plants from the Horto de Plantas Medicinais at UFLA and rooted in expanded polypropylene trays with 128 cells. After 30 days, with previously analyzed soil, the seedlings were transplanted to the field and conducted in an experimental design in randomized blocks, in a split-plot scheme, with five treatments, three replications, and eighteen plants per plot, spaced 0.25 × 0.25 cm, in two cutting times.

The treatments consisted of the soil control (T1), pure vermicompost (V1) (T2), environmental vermicompost (V2) (T3), and commercial vermicompost (V3) (T4), with the application of 6 $L \cdot m^{-2}$, in addition to conventional fertilization (T5) which consisted on the application of 80 $g \cdot m^{-2}$ of the commercial formulation of N-P-K 10-10-10.

2.5. Extraction of Essential Oils

The stems of the plants were cut at 5 cm from the ground after 95 days of transplanting when the first plants started the flowering phase. Immediately after harvesting, fresh biomass was obtained, and subsequently, the material was fractionated, placed in kraft paper bags, and taken to a forced air circulation oven at 30 °C until constant mass, thus obtaining the dry biomass of the aerial part (g).

For the essential oil (EO) extractions, the dry biomass of the aerial part was cut into small pieces, weighed, and placed in a 5 L volumetric flask with a round bottom. Then, deionized water was added to the flask until the plant material was submerged, allocated under a thermal blanket, and subjected to hydro distillation for 3 h with a Clevenger-type apparatus, whose procedure was repeated three times. After extraction, the essential oil was dried with anhydrous Na_2SO_4 and placed in glass jars that were stored at a temperature below 0 °C.

2.6. Statistical Analysis

To compare the results, the homoscedasticity and normality tests were previously performed using the ASSISTAT software [22]. Then, the analysis of variance of the data was conducted, by the F test, using the statistical program (SISVAR) and the Scott-Knott test of means [23] (was applied for dry biomass of the aerial part and essential oil content.

The characterization analysis of the three vermicomposts was conducted using the non-parametric Kruskal-Wallis test [24], followed by the Conover post hoc test [25], with Hommel correction [26]. The analysis of multiple comparisons at significance levels 5 and 10% were performed using R software version 3.6.3. And principal components analysis (PCA) by R software [27].

3. Results

3.1. Analysis of Chemical and Physical Parameters

The experimental results for pH, density (D), electrical conductivity (EC), organic matter (OM), carbon/nitrogen ratio (C/N), humic acid (HA), fulvic acid (FA), HA/FA ratio, polysaccharides (P), respiration (R), chemical oxygen demand (COD), sugar reduction (SR), and chemical indicators measured at the end of the vermicomposting process for the three formulations are presented in Table 2.

Table 2. Analysis of the chemical parameters evaluated in each treatment with three replicates (mean ± standard deviation).

Parameters	Pure Vermicompost (V1)	Soilborne Microorganisms Vermicompost (V2)	Commercial Microorganisms Vermicompost (V3)
pH	7.10 ± 0.00	7.00 ± 0.00	6.70 ± 0.00
Density ($g \cdot cm^{-3)}$	0.44 ± 0.00	0.46 ± 0.00	0.45 ± 0.06
Electrical Conductivity ($dS \cdot m^{-1}$)	6.24 ± 0.00	7.17 ± 0.00	5.83 ± 0.35
Organic Matter (%) *	28.67 ± 3.06	31.33 ± 4.04 *	28.67 ± 1.53
C/N Ratio	19.11 ± 2.04	17.41 ± 2.25	18.00 ± 2.06
Humic Acid (HA)($mg \cdot g^{-1}$)	11.01 ± 1.67	15.43 ± 3.01	13.43 ± 1.58
Fulvic Acid (FA) ($mg \cdot g^{-1}$)	5.33 ± 2.41	7.43 ± 0.93	9.80 ± 2.49
HA/FA Ratio ($mg \cdot g^{-1}$) **	2.25 ± 0.72	2.09 ± 0.45	1.41 ± 0.27 **
Polysaccharides ($mg \cdot g^{-1}$)	42.33 ± 9.29	44 ± 1.73	45.00 ± 6.24
Respiration $mg \cdot g^{-1}$	540 ± 85.44	586.67 ± 151.77	546.67 ± 87.37
COD $mg \cdot g^{-1}$	1992.67 ± 211.10	1736.67 ± 650.10	2049.33 ± 87.19
Sugar Reduction (%)	88.33 ± 3.51	94.33 ± 6.03	92.00 ± 7.21

(*), (**) Significant statistical difference at $p < 0.05$ * and $p < 0.10$ ** by the Kruskal-Wallis test.

The variables: pH, density, and electrical conductivity did not show any variability between observations. The organic matter content (% of carbon) showed a difference between the medians of each treatment by the Kruskal-Wallis test, being possible to infer that there is a statistical difference $p < 0.087$ of significance between the formulations. The ratio (C/N) also showed a difference between the levels, but there is no significant difference at 10%. Therefore, it can be considered that vermicomposts have statistically equivalent levels for this variable. While for the parameters HA, FA, HA/FA, P, R, COD, and SR, all treatments showed variability.

Table 3 shows the results of the analysis in mineral vermicomposts. Among the macronutrients, all have variability at some level for Commercial Microorganisms Vermicompost (V3). Calcium (Ca), magnesium (Mg), sodium (Na), and nitrogen (N) do not show variability regarding the formulation of pure vermicompost (V1) and soilborne vermicompost (V2). However, it is possible to infer a statistically significant difference between vermicompost formulations for phosphorus content ($p < 0.046$).

Table 3. Analysis of macro-and micronutrients evaluated in each treatment with three replicates (mean ± standard deviation).

Parameters	Pure Vermicompost (V1)	Soilborne Microorganisms Vermicompost (V2)	Commercial Microorganisms Vermicompost (V3)
Phosphorus (%) **	2.94 ± 0,17	2.38 ± 0.17 **	2.61 ± 0.13 *
Potassium (%)	1.84 ± 0,34	1.83 ± 0.41	1.39 ± 0.15
Calcium (%)	7.30 ± 0.00	8.80± 0.00	6.53 ± 0.49
Magnesium (%)	0.71 ± 0.00	0.88 ± 0.00	0.65 ± 0.11
Sodium (%)	0.08 ± 0.00	0.9 ± 0.00	0.67 ± 0.15
Sulfur (%)	0.41 ± 0.12	0.25 ± 0.1	0.33 ± 0.19
Total Nitrogen (%)	1.50 ± 0.00	1.80 ± 0.00	1.60 ± 0.10
Iron (ppm)	1.10 ± 0.00	1.80 ± 0.00	1.30 ± 0.17
Copper (ppm)	39.33 ± 5.51 **	37.33 ± 4.51 **	27.33 ± 5.03 **
Manganese(ppm)	241.33 ± 33.83	206.33 ± 45.32	257.33 ± 39.53
Zinc/= (ppm)	166 ± 0.00	229 ± 0.00	173.67 ± 19.73
Boron(ppm)	0.11 ± 0.04	0.06 ± 0.04	0.38 ± 0.52
Cadmium(ppm)	0.84 ± 0.62	0.11 ± 0.01	0.11 ± 0.00
Molybdenum (%)	0.21 ± 0.17	0.17 ± 0.06	0.12 ± 0.03
Cobalt (%)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Silicon(ppm)	11.00 ± 1.00	10.33 ± 2.89	9.83 ± 1.89

(*), (**) Significant statistical difference at $p < 0.05$ * and $p < 0.10$ ** by the Kruskal-Wallis test.

However, from the Conover test, with Hommel correlation, it is possible to identify a statistical difference between the pure vermicompost (V1) the soilborne vermicompost (V2), and the Commercial Microorganisms Vermicompost (V3), regarding the phosphorus levels, whose highest median was observed in Commercial Microorganisms Vermicompost (V3). Among the micronutrients, all elements showed variability for the three formulations, but only the copper (Cu) content ($p < 0.05$) differs statistically between the vermicompost formulations by the Conover test. For the other micronutrients, no variability was observed between treatments, so it is not possible to determine statistical equivalence for the variables.

3.2. Analysis of Biological Parameters

The results obtained for the amino acid contents (tryptophan, cysteine, thiamine, riboflavin, pyridoxine, folic acid, and nicotinic acid) are presented. The amino acids quantified in the vermicompost formulations showed variability in all treatments. 5% only for the amino acid tryptophan ($p < 0.032$), for the other variables there was no difference between treatments (Table 4).

While the medians obtained for the hormones (gibberellins, auxins, cytokinins, zeatin, and brassinosteroids) can be seen in Table 4. It can be seen that the levels of hormones quantified in the vermicompost formulations showed variability between all treatments. However, only the levels of zeat ($p < 0.09$) and auxin ($p < 0.07$) showed a significant difference at 10% probability. For the other variables, there was no difference between treatments.

Finally, the catalytic microbiome with the identification and quantification of the 16 species of microorganisms can be seen in Table 5. Although there is great diversity in the microbial community, only *Bacillus subtilis* ($p < 0.06$) and *Trichoderma* spp. ($p < 0.02$) showed a statistically significant difference at 10% by the Conover test, respectively, between the vermicompost formulations.

Table 4. The identification and quantification of amino acids and hormones were evaluated in each treatment with three replicates (mean ± standard deviation).

Parameters	Pure Vermicompost (V1)	Soilborne Microorganisms Vermicompost (V2)	Commercial Microorganisms Vermicompost (V3)
Tryptophan(ppm)	1.13 ± 0.35	2.23 ± 0.25 **	2.50 ± 0.40 **
Cysteine (ppm)	1.37 ± 0.42	1.90 ± 0.80	1.83 ± 1.10
Thiamine (ppm)	0.87 ± 0.21	0.93 ± 0.23	1.77 ± 1.34
Riboflavin (ppm)	6.67 ± 3.51	8.67 ± 2.89	12.67 ± 6.81
Pyridoxine (ppm)	27.00± 18.03	25.67 ± 7.77	40.00 ± 1.73
Folic Acid (ppm)	2.77 ± 3.68	1.13 ± 0.78	0.78 ± 0.38
Nicotinic Acid (ppm)	1.03 ± 0.25	0.37 ± 0.06	0.80 ± 0.50
Gibberellins (ppm)	2.87 ± 1.59	1.33 ± 0.78	1.37 ± 0.12
Auxins (ppm)	1.93 ± 0.91	2.53 ± 0.49	1.10 ± 0.44 *
Cytokinins (ppm)	1.33 ± 0.55	1.73 ± 1.01	0.70 ± 0.35
Zeatin (ppm)	0.33 ± 0.32 *	1.83 ± 0.55	0.93 ± 0.21
Brassinosteroids (ppm)	0.40 ± 0.20	1.07 ± 0.06	1.03 ± 0.64

(*), (**) Significant statistical difference at $p < 0.05$ * and $p < 0.10$ ** by the Kruskal-Wallis tests.

Table 5. Identification and quantification of microorganisms were evaluated in each treatment with three replicates (mean ± standard deviation).

Microrganisms (LOG CFU·g^{-1})	Pure Vermicompost (V1)	Soilborne Microorganisms Vermicompost (V2)	Commercial Microorganisms Vermicompost (V3)
Acinetobacter spp.	1.59 ± 0.91	2.35 ± 1.03	1.92 ± 0.36
Actinomyces spp.	1.51 ± 0.42	1.64 ± 0.68	2.01 ± 0.43
Azotobacter chroococcum	0.83 ± 0.33	1.5 ± 0.48	1.76 ± 0.44
Bacillus megaterium	0.91 ± 0.21	0.94 ± 0.28	2.16 ± 0.27
Bacillus subtilis *	0.96 ± 0.86 *	1.51 ± 0.67 *	3.12 ± 0.14 *
Bradyrhizobium japonicum	1.32 ± 0.66	1.92 ± 0.38	1.95 ± 0.96
Hypocrea spp.	1.3 ± 0.62	2.33 ± 1.45	1.64 ± 1.2
Humicola sp	1.36 ± 0.81	2.26 ± 1.22	2.55 ± 0.48
Mycelia sterilia	1.31 ± 0.32	2.1 ± 1	2.01 ± 0.83
Nitrosomonas spp.	2.14 ± 0.88	2.33 ± 0.67	1.47 ± 0.67
Nitrospira spp.	1.55 ± 0.5	1.95 ± 0.59	1.9 ± 1.13
Pseudomonas fluorescens	2.18 ± 1.11	2.6 ± 0.52	2.77 ± 0.93
Streptomyces spp.	2.19 ± 0.74	2.16 ± 0.72	3.26 ± 0.94
Trichoderma hamatum	2.41 ± 1.07	2.15 ± 0.13	2.12 ± 1.6
Trichoderma spp.*	1.24 ± 0.38 *	1.59 ± 0.39 *	2.43 ± 0.2 *
Thiobacillus spp.	1.33 ± 0.46	1.98 ± 1.02	2.77 ± 1.05

(*) Significant statistical difference at $p < 0.05$ * by the Kruskal-Wallis tests.

All parameters characterized for the three vermicomposts formulated in this research were compared to the specifications of IN n° 61 of 07/08/20; Art. 9 [28] (Table 6). Even though for some parameters, no significant difference was observed between treatments, the three vermicompost formulations presented chemical, physical, and biological parameters above the minimum required by law.

Table 6. Minimum guarantees for products with secondary macronutrients and/or micronutrients for application in soil, leaves, and hydroponics, according to Art. 9 IN No. 61 of 07/08/20 (continued).

Minimum Total Content For Soil Application				
Parameters	%			
	IN n° 61	Pure Vermicompost (V1)	Soilborne Microorganisms Vermicompost (V2)	Commercial Microorganisms Vermicompost (V3)
Nitrogen (N)	1.00	1.50	1.80	1.60
Phosphorus (P_2O_5)	1.00	2.94	2.33	2.61
Potassium (K_2O)	1.00	1.83	1.82	1.39
NPK	5.00	6.27	5.95	5.60
Calcium (Ca)	1.00	7.30	8.80	6.30
Magnesium (Mg)	1.00	0.71	0.88	0.65
Sulfur (S)	1.00	0.27	0.19	0.37
Ca + Mg + S	3.00	8.28	9.87	7.32
Chlorine (Cl)	0.10	-	-	-
Cobalt (Co)	0.005	0.10	0.10	0.10
Nickel (Ni)	0.005	<0.20	<0.20	<0.20
Molybidenum (Mo)	0.005	0.20	0.17	0.12
	%	ppm		
** Manganese (Mn)	0.02	241.33	206.33	257.33
** Zinc (Zn)	0.10	166.00	229.00	173.67
** Boron (B)	0.10	0.10	0.02	0.07
Iron (Fe)	0.02	1.10	1.80	1.30
** Copper (Cu)	0.02	39.33	37.33	27.33
*** Cadmium (Cd) (mg/kg) (maximum)	3.00	0.84	0.10	0.11
Silicon (Si)	0.05	11.00	10.33	9.83
		%		
Organic Carbon	15.00	28.67	31.33	28.60
C/N Ratio (Maximum)	20.00	19.11	17.41	18.00
pH	6.00	7.10	7.00	6.70
Total Nitrogen	0.50	1.50	1.80	1.60
CTC	-	-	-	-
* Free Aminoacids	5.00	40.83	40.90	60.35
* Humic Acid	15.00	11.30	18.30	14.80
* Fulvic Acid	3.00	3.80	8.20	9.30

* Minimum amounts to be classified as biofertilizers; ** Boron tolerance (B), up to 2 (two) times the declared content; for Copper (Cu), Manganese (Mn), and Zinc (Zn), up to 3 (three) times the declared content of these nutrients; *** Maximum limits of contaminants allowed in organic fertilizers and soil conditioners (SDA No. 27, 5 June 2006, Amended by IN SDA No. 7, of 4 December 2016).

3.3. Statistical Correlation of the Characterization of Vermicomposts

It is verified that in vermicompost without inoculation (V1) and vermicompost with soilborne microorganisms (V2) there was a strong positive association between *Actinomyces* spp. and humic acid with correlation coefficients 0.99 and 0.85. In Commercial Microorganisms Vermicompost (V3) the association between these variables was negative (Annex 1). In the vermicompost with the inoculation of soilborne microorganisms (V2) the correlation was significant, that is, the presence of *Actinomyces* spp. influenced the increase in humic acid content. Inoculation also positively influenced the association between *Nitrosomonas* spp. and the content of gibberellins and auxin, with an opposite force for cytokinin and zeatin.

As for the association of microorganisms and hormones for the Commercial Microorganisms Vermicompost (V3), there are positive associations, but with less force, and only for brassinosteroids, there is a strong and positive association with *Azotobacter chrooccocum*, *Bacillus megaterium*, and *Bacillus subtilis*. While the pure vermicompost (V1) presents a

strong positive correlation between the zeatin and auxin contents with the population of Bacillus subtilis.

For nutrient content, it is found that *Bacillus megaterium*, *Bacillus subtilis*, and *Azotobacter chrooccocum* are correlated to the content of potassium and iron, and *Nitrospira* spp. to the nitrogen content for Commercial Microorganisms Vermicompost (V3). While the phosphorus content for the vermicompost without inoculation (V1) has a strong positive association with *Actinomyces* spp. and *Azotobacter chrooccocum*, for the species *Pseudomonas fluorescens* there is a negative correlation, that is, their presence reduces the phosphorus content to this formulation. Moreover, *Nitrosomonas* spp. is highly correlated with the sulfur content in the soilborne vermicompost (V2).

The presence of *Actinomyces* spp. and *Azotobacter chrooccocum* are highly negatively correlated with the cadmium content, that is, the presence of these bacteria by inoculation of soilborne microorganisms in the vermicompost (V2) can reduce the presence of toxic metals. These same bacteria for the uninoculated vermicompost (V1) are strongly associated positively with the silicon content, which gives the plants resistance to the attack of pathogens.

In general, regardless of the vermicompost formulation, the bacteria *Azotobacter chrooccocum*, *Bacillus megaterium*, and *Bacillus subtilis* have a great influence on the parameters evaluated such as minerals, hormones, and amino acids.

3.4. Principal Components Analysis

According to Kaiser's criteria, the main components (PC1, PC2, and PC3) are adequate to explain the relationship between the three vermicompost formulations and the community of microorganisms, as well as the content of minerals (macronutrients) (Figure 2A,B) and (Table 7)

Table 7. Principal components, eigenvalues, and percentage of variance are explained by components.

Principal Component Microrganism	**Eigenvalue**	**Percentage of Variance**	**Cumulative Percentage of Variance**
PCA1	5.154	32.213	32.213
PCA2	3.229	20.181	52.395
PCA3	2.588	16.176	68.571
PCA4	1.801	11.257	79.829
PCA5	1.374	8.590	88.419
PCA6	0.924	5.777	94.197
PCA7	0.707	4.422	98.619
PCA8	0.220	1.380	100
Principal Componente Macronutrients	**Eigenvalue**	**Percentage of Variance**	**Cumulative Percentage of Variance**
PCA1	3.938	56.259	56.259
PCA2	1.403	20.056	76.316
PCA3	1.123	16.054	92.370
PCA4	0.321	4.593	96.964
PCA5	0.190	2.717	99.681
PCA6	0.019	0.281	99.963
PCA7	0.002	0.036	100

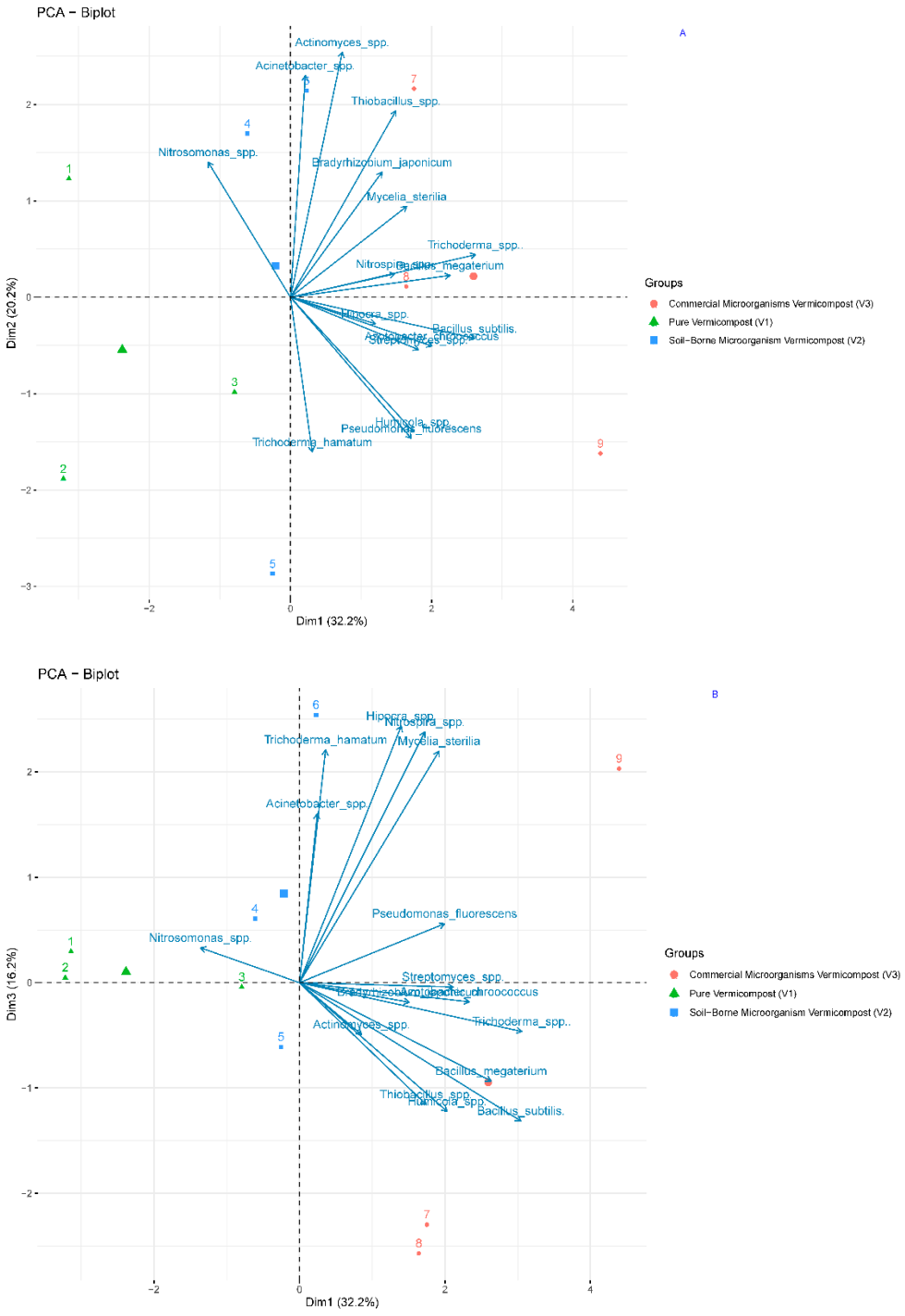


Figure 2. Principal components analysis for microbial assessment (**A**) and (**B**) of the three vermicomposting formulations. 1: vermicompost without inoculation (V1); 2: soilborne microorganisms vermicompost (V2); 3: commercial microoganisms vermicompost (V3).

For the analysis of microorganisms present in vermicompost formulations, PC1 and PC2 (Figure 2A,B and Table 7) explain 52.39% of the variation between treatments, while the cumulative percentage of PC1, PC2 and PC3 (Table 7), explains 68.57% of the variation. Principal components (Figure 1a,b) show strong positive loadings (>0.7) for *Acinetobacter* spp. *Actinomyces* spp., *Bacillus megaterium*, *Bacillus subtilis*, *and Trichoderma* spp. Higher concentrations of *Hypocrea* spp., *Acinetobacter* spp., *Nitrospira* spp. *Mycelia* spp.

Mycelia sterilia, *Armillaria* spp., *Streptomyces* spp., and *Pseudomonas fluorescens* in vermicompost from soilborne microorganisms (V2) (Figure 1a).

For the Commercial Microorganisms Vermicompost (V3), a greater presence of *Bacillus megaterium*, *Bacillus subtilis*, *Triobacillus* spp. *Trichoderma* spp. and *Actinomyces* spp. and *Bradyrhizobium japonicum* (Figure 1b). When the vermicompost was not inoculated in any way, in the Pure Vermicompost treatment (V1), less microbial diversity was observed in its composition, with a possible greater population of the genus *Nitrosomonas* spp.

To explain the relationship between vermicompost formulations and macronutrient contents (Figure 3), it appears that the two components together explain 86.4% of the total variation, with the first principal component (PC1) explaining 56.3% of the total variation, with strong positive loadings (>0.7) for nitrogen, calcium, magnesium, sodium, and total nitrogen contents and strong negative loading (<−0.7) for phosphorus.

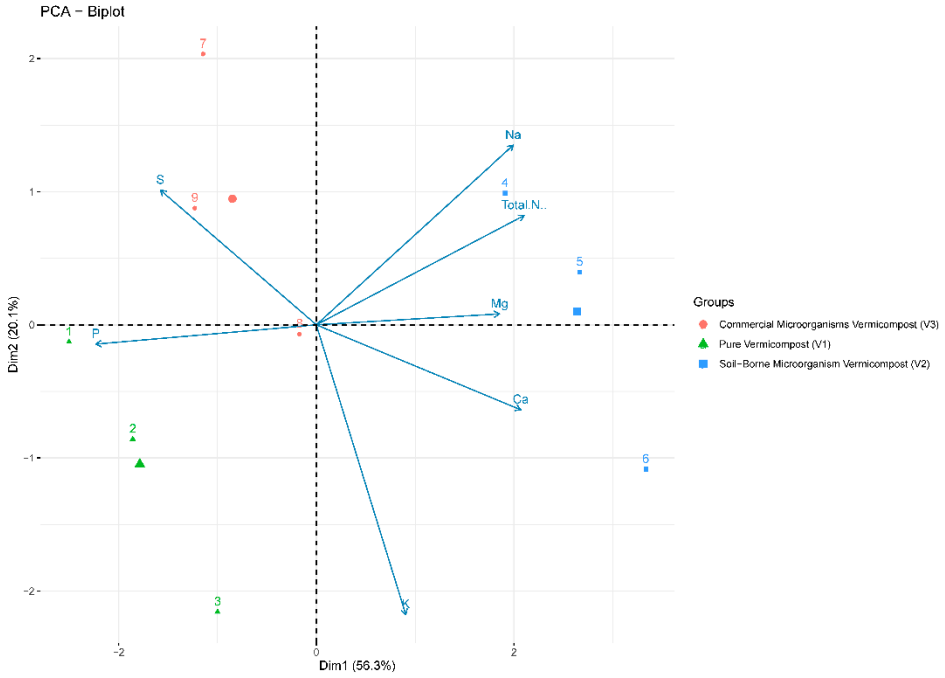


Figure 3. PCA of the macronutrients of the three vermicompost formulations. 1: vermicompost without inoculation (V1); 2: soilborne vermicompost (V2); 3: commercial vermicompost (V3).

As for the behavior of the macronutrient contents concerning the formulations, it appears that the Pure Vermicompost (V1) presented a higher concentration of P_2O_5 when compared to the others. While the soilborne vermicompost (V2), inoculated with efficient microorganisms from the native forest, highlighted the nutrients Ca Mg, total Nitrogen, Na, and K_2O, and finally, the Commercial Microorganisms Vermicompost (V3), presented concentrations of S, more evident, reinforcing the influence of the microorganisms in the dynamics of the highlighted nutrients.

3.5. Application of Vermicompost in the Production of Origanum vulgare L.

Nutritional management (vermicompost and N-P-K) and cutting time showed significant interaction at $p < 0.01$ (Table 8). For all treatments applied in the field, it was verified that the second cut presented a higher production of dry biomass in the shoots (g/m^2) and essential oil content (%OE). Oregano is considered a perennial plant, and as a result of this factor, greater productivity is expected in the second cut compared to the first.

Table 8. Production of dry biomass (g/m^2) (FS) of the aerial part and essential oil content (%) influenced by the application of three formulations of vermicompost in comparison with the controls (soil and NPK), in two cutting seasons.

Tratamentos	Dry Mass				Essential Oils			
	——— g ———				——— % ———			
	1° cut		2° cut		1° cut		2° cut	
Soil (T1)	26.66	aA	147.50	aB	0.208	aA	0.278	aB
Pure Vermicompost (T2)	35.00	aA	192.00	bB	0.375	cA	0.270	aB
Soilborne Vermicompost (T3)	43.00	aA	232.50	cB	0.228	aA	0.425	bB
Commercial Vermicompost (T4)	33.00	aA	261.66	dB	0.327	bA	0.303	aB
NPK (T5)	71.66	bA	253.33	dB	0.508	dA	0.360	bB

Treatments followed by equal letters, lowercase in the column and uppercase in the row, do not differ significantly by the Scoot-Knoot mean test at $p < 0.01\%$.

4. Discussion

4.1. Dynamics of pH, Density, and Electrical Conductivity

At the end of the vermicomposting processes in all treatments (V1, V2 e V3), the pH values were in the neutral range, ranging from 7.1, 7.0, and 6.7, respectively, and all were above the minimum required by legislation. Density also showed no variability between treatments, with a median of 0.45 (kg/cm^3). During vermicomposting, the organic substrate is fragmented, increasing the ratio of small particles, especially those smaller than 5 mm, modifying the apparent density of the material. This mechanical action increases the surface-volume ratio of the aggregate particles, thus increasing the microbial activity on the organic substrate [29].

Electrical conductivity (dS·m^{-1}) is associated with the solute content dissolved in the vermicompost [30]. The formulations showed variability in the contents with 6.24 (dS·m^{-1}) for pure vermicompost (V1), 7.17 (dS·m^{-1}) in the soilborne vermicompost (V2) and 5.83 (dS·m^{-1}) for the Commercial Microorganisms Vermicompost (V3).

Electrical conductivity (EC) is related to the mineralization of organic material by earthworms, microorganisms, and the levels of soluble salts [31]. In "vermicomposted" cattle manure, EC presented an average of 2.51 and 2.91 (dS·m^{-1}), lower values than observed in this experiment, while [29].

The reduction in pH close to neutral, in the range of 6.5 and 7.8, is reported by many researchers using different types of organic waste and proportions of cattle manure in the vermicomposting process [30–33]. According to Cotta [34] the combined effect of oppositely charged ions (ammonium and humic acid groups) formed in the bio-oxidation of organic material, acts in the regulation of pH towards neutrality.

Regardless of the formulation, the levels of soluble salts and pH (Table 2) are within the agronomic limits stipulated by IN No. 61 of 07/08/20 (BRASIL, 2020). These parameters indicate that at the end of the vermicomposting process, the final product reached full maturation, being able to be applied in the field in the cultivation of plant species, corroborating Souza [35] by using the pre-composting method, followed by vermicomposting of urban pruning, sewage sludge, and organic waste.

4.2. Dynamics of Organic Matter and Carbon/Nitrogen Ratio

Organic matter content (% of carbon) and carbon/nitrogen ratio (C/N) are highly correlated variables regarding the degree of maturation of vermicompost, contributing to the increase in soil organic matter content, supply of nutrients, and a favorable microclimate for the development and multiplication of microorganisms in the rhizosphere.

Alidadi [33], evaluating the recycling of organic waste by vermicomposting, in different formulations, found a C/N ratio in the proportions of 14.01, 12.93, 16.61, and 28.16. The result corroborates the proportions found for this study, whose C/N ratio for the three

formulations, with and without inoculation of efficient microorganisms, showed a variation of 19.11 (V1), 17.41 (V2), and 18.00 (V3).

The pure vermicompost (V1) showed higher variation in the % of OM, with contents in the range of 31%, corroborating Marques [35] with values ranging around 30.10% and 38.7% of organic matter and a C/N ratio of 15/1 and 16/1. Cotta [34] evaluated the vermicomposting of vegetable residues + manure, showing a C/N ratio of 16/1 and 37.8% of OM; however, in the research carried out by Ramnarain [31], the reported values were below the aforementioned articles, with a % OM of 18.53% and a C/N ratio of 13:1.

The C/N ratio below 20:1 has been used as an indicator of the maturity of composts and vermicomposts [32] the ideal C/N ratio for vermicomposting being 25:1 [29], although it can be considered adequate between 1/20 and 1/25. The IN n° 61 of 07/08/20 recommends that the C/N ratio should be at most 20/1, corroborating the results found in this research for vermicomposting formulations.

The values for the organic matter content and C/N ratio shown in this study, in addition to being an indicator of vermicompost maturity, can be correlated with the activity of the microbial community present in the vermicomposts (Table 5), as well as the bio-oxidation carried out by earthworms in their digestive tract, where the humification process of the vermicompost will reflect in a lower C/N ratio than that of non-stabilized organic residues, thus contributing to greater availability of nutrient levels, due to mineralization of OM by earthworms [36].

In general, the C/N ratio is within the maximum limit required for class A organic compound fertilizers, indicating that this fertilizer is commercially possible [28].

The increase in soil organic matter contents due to the addition of vermicompost reflects in the restoration, restructuring, and maintenance of soil life, contributing to microbial activity, nutrient cycling, preservation, and maintenance of the ecosystem, essential to achieve sustainable agrifood systems and food security [1–3].

According to Atiyeh [37], the accelerated humification of vermicompost leads to a lower C/N ratio, which is inversely proportional to the increase in mineral nutrients (P, K, and N) due to OM mineralization by earthworms.

4.3. Dynamics and Relationship of Humic Acid and Fulvic Acid

Vermicompost contains humic substances, which are condensed organic compounds that differ from biopolymers owing to their macromolecular structure and high persistence in the soil [38]. Humic substances contain a hydrophilic part, fulvic acids, and a hydrophobic part, humic acid (HA) [39], aggregates organized into low (FA) and high (HA) molecular weight organic compounds [40].

Humic and fulvic acids are molecules that can directly or indirectly influence plant metabolism, acting on ion transport, respiratory activity, chlorophyll content, nucleic acid synthesis, and the activity of several enzymes. Furthermore, its interaction with the rhizospheric microbiome improves the assimilation of nutrients [41–44].

Even not having observed a statistically significant difference for the parameters HA and FA, there is variation between the treatments, however, for the HA/FA ratio, there is a statistically significant difference at $p < 0.06$. Thus, it is possible to infer that the soilborne vermicompost (V2) although presenting higher variability for humic acid, the HA/FA ratio did not differ from the pure vermicompost (V1). As for the Commercial Microorganisms Vermicompost (V3), there is higher variability for fulvic acid, but as for the HA/FA ratio, it is lower than the other treatments.

The inoculation of soilborne efficient microorganism to the vermicompost (V2) contributed to the highest median of humic acid, with 15.43%, when compared to the other vermicomposts with 11% and 13%, V1 and V3, respectively. According to Atiyeh [45], the process of humification of organic material occurs through fragmentation and reduction of particle size and elevation of microbial activity inside the earthworm's intestine, facilitating the humification of organic matter by the earthworm mucus.

The differences observed for the highest levels of humic acid for vermicompost (V2) and fulvic acid for vermicompost (V3) (Table 2) can be explained by the higher C/N ratio, higher carbon content and higher total nitrogen concentration for the (V2). These parameters influence the conversion of humic substances, due to the reduction in the rate of decomposition. Lower rates of decomposition of organic matter contribute to higher levels of carbon and, consequently, to higher levels of humic acid since this molecule has higher levels of C in its chemical composition than fulvic acid.

In addition, the lower total nitrogen content observed for vermicompost V3 (Figure 3) may be related to the higher consumption of this nutrient for the decomposition of the carbon present in the formulation, and, consequently, leading to lower levels of humic acid, and higher levels of fulvic acid, which depends on the transformation of organic matter, as well as to the formation of the humic acid molecule, that contains higher levels of N compared to fulvic acid [46].

For vermicompost V3, the higher consumption of N in the formulation can be explained by the larger population of Bradyrhizobium japonicum, whose action of rhizobacteria can stand out in the competition for nutrients and ecological niches, suppressing other microorganisms [47].

According to Schnitzer e Khan [48], the chemical compositions and average functional groups of humic acids and fulvic acids reveal that humic acids present more C and less O than fulvic acids, showing that humic acid is relatively more polymerized than fulvic acid and that fulvic acids contain more acidic functional groups per unit mass than humic acids.

The action of earthworms is known to occur in symbiosis with the microorganisms present in the residue to be bio-oxidized, and the inoculation of efficient microorganisms in the formulation (V2) may have contributed to the increase in the activity of microorganisms on the mass to be transformed.

Corroborating this study, Hervas [49] evaluating six vermicompost formulations found humic substances contents ranging from 3.6% in urban solid waste to 17.2% with cow manure, found values below reported in this research with values around 6% [34]. The contents of humic substances in vermicomposts have been associated with beneficial effects in plant cultivation, regarding the elongation and formation of lateral roots, improvement of the microbial structure of the soil and root nodulation, as well as in mycorrhizal colonization [7]. Studies comparing the application of vermicompost and the same amount of humic and fulvic acid alone found equal effects between vermicompost and the specific product alone [50].

4.4. Polysaccharide Dynamics, Respiration, Biochemical Oxygen Demand (BOD), and Sugar Reduction

The respiration rate, sugar reduction, and biochemical oxygen demand are related to microbial activity in the decomposition of organic material and influence the time taken for the final maturation of the product, as well as its stabilization. In addition to serving as a measure of the degradable mass load that can be converted into bio-gas, a growing trend in the current scenario [51]. There is a relationship between the respiration rate in humification and nitrogen transformation, indicating high levels of nitrification and denitrification. BOD correlates with sugar reduction, generating energy for microorganisms to transform organic material [34,52].

The respiration rate of the vermicompost at the end of maturation ranged from 540 $mg \cdot g^{-1}$ to 586 $mg \cdot g^{-1}$, the lowest variability in biochemical oxygen demand was observed for the vermicompost inoculated with soilborne microorganisms (V2), with 1736.66 mg^{-1} and a greater variability for Commercial Microorganisms Vermicompost 2048.33 $mg \cdot g^{-1}$.

4.5. Dynamic of Nutrients

The potential for releasing nutrients into the soil through the addition of organic residues, either as soil conditioners or organic fertilizers, for the recovery of degraded soils or plant nutrition, is a promising low-cost and viable social technology for family

farmers [53]. Among the macronutrients, the phosphorus content showed a tendency to increase, and a statistical difference was observed at $p < 0.05$. The pure vermicompost (V1) presented higher phosphorus content when compared to the commercial (V3) and soilborne (V2) vermicompost formulations, with a median of 3%, 2.6 and 2.3% respectively.

Due to the activity of the phosphatase enzyme in the gastrointestinal tract of earthworms, an increase in phosphorus content may occur due to mineralization by bacteria and enzymes present in the bio-oxidation and maturation process [54]. In the present work, the reduction in the phosphorus content observed for the treatments with the addition of soilborne microorganisms (V2) and commercial microorganisms (V3), may be due tothe increase of the microbial community in the organic mass, leading to the immobilization of the P, and higher energy consumption for carbon decomposition.

Contrary to what is imagined, microorganisms do not consume only nitrogen to perform the humification of organic material, studies show that the carbon/phosphorus ratio influences the P mineralization process and that when using P in microbial metabolism may be immobilized due to the mineralization of orthophosphate ions. Both processes occur simultaneously and influence the greater or lesser release of phosphorus [55].

For the other macronutrients, although there was no statistical difference between the vermicompost formulations, all treatments showed nutrient levels above the minimum requirement described by Brazilian legislation in IN No. 61 of 07/08/20 (Table 6).

The potassium and nitrogen contents, although they did not differ in the treatments, presented contents on average of 1.68% and 1.6%. While the calcium contents were on average 7.54%, magnesium 0.75%, sodium 0.5%, and sulfur 0.3%.

Among the micronutrients, only the copper content differed statistically between treatments, with the highest content for pure vermicompost (V1). For iron, zinc, and cadmium, no variation was observed between the different types of vermicompost. For manganese contents, the treatment (V3) presents greater variability. As for silicon and molybdenum, the highest variability is observed for (V2), and for copper, it is possible to infer that there is a statistical difference between treatments, where (V3) has the lowest contents compared to (V1) with the highest concentration.

It is known that the application of vermicomposting to the soil can result in effects on mineral nutrition, phytotoxicity, and supply of mineral elements to cultivated species and slowly and gradually aid in the prevention of nutrient leaching and/or percolation. And even if some metals are immobilized by the action of microorganisms, the remobilization of elements can occur due to changes in soil redox potential, pH, and the presence of humic substances that influence soil fertility [56]. In general, similar results regarding the characterization of macro and micronutrients, using different organic residues, corroborate this research [57].

Fertility is generally evaluated by mineral analysis of macro and micronutrients, C/N ratio, and cation exchange capacity (CEC) [58] where the negative charges, responsible for the increase in CEC, are present in the carboxylic functional groups (-COOH), phenols (-OH), alcohols (-OH) and methoxylic (-OCH3) of organic acids present in humic substances, organic matter content, depending on the pH of the medium.

Therefore, although the three formulations present excellent chemical characteristics and can be applied in the form of soil conditioner or compound organic fertilizer, according to Brazilian legislation, the exclusive elemental measurement of nutrients present in vermicompost formulations, are not isolated factors. That will reflect or converge in the greater or lesser release of nutrients to the soil solution, and consequently, absorption, immobilization, or leaching of these elements, on the contrary, must be analyzed together, observing other parameters that are directly correlated to soil fertility, such as the presence of microorganisms, hormones, humic substances, among others.

4.6. Dynamics of Amino Acid Content

The amino acids quantified in the vermicompost formulations showed variability in all treatments. There is greater variability of tryptophan (ppm) in the formulations with

soilborne (V2) and commercial (V3) microorganisms, with a statistical difference at <0.05. For cysteine, no variation between formulations is observed.

The higher tryptophan content for vermicompost V2 can be explained by the higher humic acid and auxin content observed in this formulation. Because humic substances functions as auxin receptors, provoking its synthesis and subsequent transcription of the responsive gene, moreover, tryptophan is a precursor compound of auxin. Thus, the inoculation with efficient microorganisms (V2), with a significant population of Bacillus subtilis and Pseudomonas fluorescens, may have contributed to the increase in the tryptophan precursor content, and, consequently, in the auxin content for this formulation [58].

For thiamine (ppm), riboflavin (ppm) and pyridoxine (ppm) the commercial microorganisms (V3) provided greater variability than the other formulations. For folic acid, although the pure vermicompost (V1) showed greater variability, the median between treatments was similar. As for the nicotinic acid content (ppm), (V1) had the greatest variability, above 0.8 ppm, and V2 the lowest, with levels below 0.4 (ppm). There are several hypotheses regarding the effects of amino acids on plant development. The main functions would be: protein synthesis; acting as an intermediate compound of endogenous plant hormones; and, complexing effect on nutrients and other agrochemicals [59].

Although it is difficult to isolate the effect of amino acids when they are present in formulations that contain macro and micronutrients, among other substances, few scientific works elucidate and quantify their levels in vermicomposts. Kudoyarova [59], emphasize that amino acids can act in the physiological processes of the plant as precursors of endogenous hormones or enzymes, such as tryptophan, which is known as a precursor of indoleacetic acid (auxin). And that studies under tropical conditions regarding the influence of amino acids on plant development are necessary.

4.7. Dynamics for Hormone Content

Only the levels of zeatin (ppm) and auxin (ppm) have a significant difference. In general, the soilborne vermicompost (V2) to which efficient microorganisms from the riparian forest were inoculated, showed the highest levels of auxin and zeatin.

As previously mentioned, the auxin content for the vermicompost (V2) is related to higher levels of humic acid, tryptophan, and the presence of rhizobacteria such as Bacillus subtilis and *Pseudomonas fluorescens*. In addition, the genus Bacillus also contributes to the production of the hormone zeatin, which is a type of cytokinin commonly produced by this genus [60]. In this context, the increase in the microbial population through the inoculation of efficient microorganisms from the native forest directly contributed to the increase in the content of phytohormones present in this formulation.

For the pure vermicompost (V1) there is greater variability in the auxin content than in the Commercial Microorganisms Vermicompost (V3) with inoculation of commercial microorganisms. While for zeatin (ppm) the formula (V3) has higher levels than (V1).

Even though there is no statistical difference between the three vermicompost formulations for this parameter, the quantification and characterization of phytohormones are important for understanding the potential and influence that vermicomposts can exert in promoting plant growth. Phytohormones are responsible for the regulation of several vital physiological functions and are cited in several studies, but the works that quantify and identify the groups of hormones present in the vermicomposting process are still scarce.

The contents of cytokinin (ppm), gibberellin (ppm), and brassinosteroids (ppm) had great variability between the formulations, showing that for brassinosteroids, pure vermicompost (V1) has lower variability, below 0.6 (ppm) compared to soilborne vermicompost (V2) and Commercial Microorganisms Vermicompost (V3). However, the highest variability of gibberellin (ppm) is observed for V1, with levels above 2.0 (ppm) and for cytokinin levels, V3 has the lowest variability, below 1.0 (ppm).

Analyzing the phytohormone content by two extraction techniques in vermicomposting of organic waste with *Perionyx excavatus* and *Eisenia fetida*, pH around 7.4, and 0.39 $S \cdot m^{-1}$ of electrical conductivity, identified the following groups of phytohormones:

Abscisic acid 0.53%; Auxin: indole-butyric acid 0.72% and indole-acetic acid 0.09% and naphthoxy-acetic acid 1.02%; Cytokinin: N-5-benzyladenine 0.21%, zeatin 2.07%, Isopentenyladenosine 0.65% [60]. The levels found for zeatin corroborate those identified in this study for the three formulations (V1, V2, and V3), but the other phytohormones are above the percentages quantified by Zhang [61].

Modulation of plant hormone levels such as auxin, cytokinin, gibberellins, brassinosteroids, and other biologically active compounds may be associated with microbial regulators [62]. Phytohormones directly impact plant metabolism by acting as growth regulators and modulators of plant response mechanisms to biotic and abiotic stress [9].

In several studies carried out with bacterial isolates, researchers observed that *Bacillus*, *Azospirillum*, *and Pseudomonas* produced cytokinins and that they stimulated the development of plant roots [63], species such as *Bacillus pumilus*, *Bacillus licheniformis*, *Acetobacter* spp., *Bacillus* spp., *Azospirillum* spp. were found among gibberellin-producing strains and that species of actinobacteria, actinomycetes, and Streptomyces produce gibberellins [64,65].

Studies also found that Enterobacter was able to produce auxin and improve corn growth in Cd-contaminated soil [64] as well as Azospirillum species can release zeatin (cytokinin), a phytohormone responsible for gene expression in tissue and organ senescence, increase in cell longevity [66] as it is involved in the regulation of nitric oxide biosynthesis, which is related to plant senescence and defense, corroborating with the results observed in this research.

Although the interaction between brassinosteroids and auxins is considered relevant in the modulation of stress resistance, xylem differentiation, antioxidant activity, root elongation, cell expansion, photosystem II efficiency, and gas exchange, only auxins, gibberellins and cytokinins are included in commercial formulations of biostimulants [67–71].

In this sense, through the various studies, there is a correlation between the quantification of phytohormones and some genera of microorganisms (Annex 1), and the phytohormones characterized in the vermicomposts (V1, V2, and V3) can contribute to the growth promotion of plants, in addition to playing an important role in mitigating the abiotic stresses that may occur.

4.8. Microbiome Characterization

The total diversity of microorganisms (log CFU·g^{-1}) quantified in the three vermicompost formulations, through the catalytic biogram expresses the potential of vermicomposting to reuse solid waste as a social technology. (Table 4). Sixteen species of microorganisms were identified, including fungi and bacteria described in the literature as plant growth promoters by the most diverse direct and indirect mechanisms [9,72], among other extremely important characteristics for the advancement of sustainable agriculture.

Among the microorganisms identified and quantified, only the bacterial population of *Bacillus subtilis* (Log CFU·g^{-1}) and fungi of the genus *Trichoderma* spp. (log CFU·g^{-1}) showed a statistically significant difference of $p < 0.06$. The Commercial Microorganisms Vermicompost (V3) showed greater variation in the population of Bacillus and Trichoderma sp. than the other formulations. The soilborne vermicompost (V2) has an intermediate population for both, while the pure vermicompost (V1) has the lowest variability.

The results indicated that the inoculation of efficient microorganisms collected in the riparian forest and commercial inoculant for the vermicompost formulations (V2) and (V3) promoted an increase in the total microbial community, with populations of 31.31 and 35.81 (log CFU·g^{-1}) respectively, when compared to the vermicomposting process without inoculation (V1), obtaining a population of 24.12 (log CFU·g^{-1}).

Studies show that in the process of bio-oxidation of organic waste, the digestive tract of earthworms, in the excretion of coprolites, can benefit some microorganisms to the detriment of others, as is the case of the genus *Azotobacter*, which is favored by coprolites [73]. This hypothesis corroborates the results observed between the difference in the population of microorganisms between the commercial vermicompost (V3) and the

soilborne vermicompost (V2), where a greater presence of *Azotobacter* was observed for the vermicompost (V3).

Byzov [74] states that the inoculation promoted by the food source is the main conditioner of the change in the microbial community, which is also influenced by the C/N ratio [75].

As the vermicomposts were inoculated with different sources (soilborne and commercial inoculant microorganisms), the low stability of the environmental microorganisms (V2) compared to the commercial one (V3), contributed to specific characteristics of the earthworms' food source, which during the ingestion process, digestive enzymes can degrade fungi and bacteria, which allows some organisms to proliferate, while others die during the process [76].

In general, the microbial community undergoes modification during passage through the intestine, with the greatest impact being on the fungal microflora [77]. For the vermicompost (V3), with a larger microbial community, a greater presence of bacterial genera was observed, such as *Bacillus megaterium*, *Bacillus subtilis*, *Triobacillus* spp., *Actinomyces* spp. and *Bradyrhizobium japonicum*. While for the soilborne vermicompost (V2) a greater presence of *Hypocrea* spp., *Mycelia* spp. *Mycelia sterilia* and *Armillaria* spp. Researchers claim that the modification of the microflora may be due to different responses of microorganisms under the influence of the intestinal fluids of the earthworm, which would be an ideal habitat for N2O-producing bacteria [78].

Therefore, the selective activity of the earthworm intestine and the decrease in diversity during the passage of ingested material through the intestine in the vermicompost (V2) is the result of the elimination of some bacterial groups. The elimination of these groups may have occurred due to several factors such as the use of bacteria as a food resource, non-adaptation of bacteria to a new environment, and elimination of certain bacterial groups to regulate the steady state of the microbial community according to [74]. and Although there was a significant difference between the vermicomposts only for Bacillus bacteria and Trichoderma fungus, for all treatments, higher microbial richness was observed in the formulations. Corroborating this study, Pathma [8] reported the presence of different strains of bacteria with potent antagonistic and bio-fertilizing potential in the vermicompost produced by *Eisenia fetida*, in bacteria belonging to the genera *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Microbacterium*, *Paenibacillus*, *Pseudomonas*, *Rheinheimera*, *Rhodococcus e Stenotrophomonas*.

Among the 16 genera identified, the actinobacteria *Streptomyces* spp. is reported to be involved in the stabilization of different organic materials when processed by *E. fetida*, as well as in the biocontrol of soil pathogens [79,80]. Observed that *Bacillus subtilis*, and *Pseudomonas fluorescens*, contribute to the growth and development of plants through the formation of chelating agents (siderophores) that have a high affinity for iron in the soil and make it more accessible to the plant and less accessible to pathogens. Iron is an important mineral in the synthesis of chlorophyll, maintaining the structure and function of the chloroplast, for the functioning of several enzymes, such as cytochromes in electron transport and photosynthetic activity.

The bacteria *Azospirillum* spp., *Bradyrhizobium* spp., and *Azotobacter chrooccocum* help in the uptake and assimilation of NH_4 and NO_3 and produce nitrogenase enzymes, which act in the fixation and subsequent supply of nitrogen to plant species [81]. Other genera of free-living N_2-fixing bacteria, such as *Nitrospira* spp. and *Nitrosomonas* spp. are also extremely important for the nitrogen cycle, acting in the ammonification and nitrification processes.

The filamentous fungi *Humicola* sp. and *Hypocrea* spp. of the genus *Trichoderma* contribute to the decomposition of organic matter and enzymatic activity in the formation of humic substances. On the other hand, fungi of the genus *Trichoderma* spp. and bacteria such as *Enterobacter* spp. can solubilize phosphates, producing organic acids that lower the pH or chelate mineral ions, releasing P, through the secretion of phosphatases and phytases [81–83].

The fungus of the genus *Trichoderma* has been reported in studies regarding its ability to suppress soil pathogens. As well as *Bacillus subtilis* it is antagonistic to nematodes and enhances plant growth [70]. In addition, about 49% of bacteria isolated from the vermicompost of *E. fetida* belong to the genus *Pseudomonas* and *Bacillus* and have a strong antagonistic potential against pathogenic fungi, such as *Bipolaris oryzae*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Cylindrocladium floridanum*, *C. scoparium*, *Fusarium oxysporum*, *Macrophomina phaseolina* [8].

The genera *Rhizobium*, *Azospirillum*, *Bacillus e Pseudomonas* are also reported to improve osmoregulation processes in resistance and tolerance to drought and salinity. According to [8] *Bacillus megaterium* relieves nickel (Ni) stress by the increased antioxidant enzymatic activity of ascorbate peroxidase, catalase, peroxidase, and superoxide dismutase, along with increased production of flavonoids, phenols, and proline.

Therefore, it is a consensus among many researchers that vermicomposting provides the appearance of beneficial microorganisms for plant growth, by contributing not only to mineral nutrition through mineral phosphate solubilization and nitrogen fixation, but also by increasing microbial activity in the soil, phytohormone secretion, enzyme and siderophore production, influencing systemic resistance to pathogens, abiotic and biotic stress [5].

However, research focused on understanding the microbial diversity of organic fertilizers such as vermicompost will be prerequisite in the future to better understand the influence on primary and secondary metabolism of plants, their influence on growth and development, as well as the interaction between vermicompost–soil–plant in the recovery and restoration of degraded soils for the advancement of sustainable agrifood systems.

4.9. Biomass Production and Essential Oil Content of Origanum vulgare L.

It is observed that in the first cut the highest dry biomass content of the aerial part was observed for the control (NPK), whereas the EO content, higher percentages can be observed for the application of NPK (T5), followed by *soilborne* Microorganism, Vermicompost (T3) and Commercial Microorganisms Vermicompost (T4), with 0.50, 0.37 and 0.32%, respectively. However, for the second cut, the highest EO content was verified in the application of *soilborne* vermicompost (T3) and NPK (T5), with 0.42 and 0.36% respectively. Noting that the vermicompost with inoculation of efficient environmental microorganisms can be applied instead of NPK in the production of *Origanum vulgare* L.

For the first cut, the higher production of dry biomass and essential oil content for the application of NPK (T5) occurs because synthetic chemical fertilizers are highly soluble and release nutrients more quickly into the soil solution, so the oregano crop was responsive to nutritional increment, reflecting in higher percentages. However, for the second cut, the higher EO content (%) found for the application of vermicompost with inoculation of microorganisms collected in the forest (3), occurs since organic fertilizers are characterized by a slow and gradual release of nutrients into the soil solution.

In addition, *soilborne* vermicompost (T3) acts not only as a fertilizer but as a soil conditioner, by increasing the organic matter content, providing humic substances, amino acids, and phytohormones, favoring the microbiological activity of the soil, due to the high microbial community present in its formulation, which can be attributed to the inoculation with efficient microorganisms from the local native forest, from the same edaphoclimatic region, where *O. vulgare* L. was cultivated in the field.

Differences in biomass production and essential oil content between the first and second cuts of *O. vulgare* L. (Table 7) can be explained by variations in nutritional management; that is, the NPK formula (T5) when applied individually and exclusively to the soil, was readily available to the crop, and part of which was absorbed and part of which may have been leached. The soilborne vermicompost (T3) provided not only macro and micronutrients for the plants but also lead to an increase in the organic matter content of the soil, which allowed the adsorption of mineralized nutrients, resulting in lower losses by leaching and also a slow and gradual release of nutrients, favoring an increase in the

content of biomass and essential oil. Through degradation and mineralization, the vermicompost contributes to the increase of the contents of essential nutrients such as nitrogen, phosphorus, and potassium, and micronutrients, iron, manganese, and zinc, among others, which are assimilated by the root system during the phenological cycle. This may be related to the good results observed in the EO production by the treatments with vermicompost since the Essential oils are molecules formed from the biosynthesis of photoassimilates, which require ATP and NADPH, products obtained from photosynthesis, which need phosphorus and nitrogen for their formation.

Another important factor that can influence plant growth, as well as EO production, is that the vermicompost also has humic substances, phytohormones, and plant growth-promoting microorganisms [62], in addition to high porosity, water retention capacity, and aeration [84], forming nutrient complexes that are gradually released to the roots of plants [85].

Studies with *O. vulgare* L. [85], *Mentha piperita* L., *Rosmarinus officinalis* L. [86], and *Ocimum basilicum* L. [87] showed greater efficiency in plant growth with the application of vermicompost when compared to synthetic fertilizer (NPK) or control (soil), emphasizing that synthetic fertilizer can be replaced by vermicompost in the cultivation of medicinal species.

Therefore, organic fertilizer can be a substitute for synthetic fertilizer in the sustainable cultivation of medicinal plants, influencing the production of biomass, as well as the content and quality of essential oil [86,87].

5. Conclusions

All vermicompost formulations meet the minimum requirements stipulated by Brazilian legislation for the registration of class A organic compost fertilizer. Based on this study, it is possible to conclude that the method of inoculation of native and commercial microorganisms in the vermicomposting process increases the levels of macro and micronutrients, the content of phytohormones, amino acids, humic substances and enriches the microbial community, regardless of the formulation. Additionally, inoculation with efficient microorganisms from the native forest promoted a greater population of *Hypocrea* sp., *Mycelia* spp. *Mycelia sterilia* and *Armillaria* spp., and for the commercial inoculant there was a higher population of bacteria *Bacillus megaterium*, *Bacillus subtilis*, *Triobacillus* sp., *Actinomyces* sp., and *Bradyrhizobium japonicum*. The potential application of vermicomposts as a class A organic fertilizer, in field conditions, allows us to conclude that vermicompost with the inoculation of microorganisms can be applied to replace chemical fertilizer in the production of dry biomass and essential oil content of *Origanum vulgare* L. The essential oil content increased from 0.27% in the control treatment (soil-T1) and application of vermicompost without inoculation (T2) and from 0.36% in the application of NPK fertilizer (T5), to 0.42%, with the inoculation of efficient microorganisms from the native forest (soilborne-T3). The advantage of inoculating the vermicompost with efficient microorganisms is the low cost, easy accessibility, and autonomy of the farmer for the production of *Origanum vulgare* L.

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