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Effect of Biochar on Vermicompost Production: Chemical, Biochemical, and Biological Properties

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Abstract: Farm and industrial residues must be adequately managed to avoid negative environmental implications. In this study, our objective was to evaluate (i) the impact of the co-production of vermicompost using grape bagasse and biochar (BC) on the yield and biochemical, chemical, and biological properties of vermicompost; (ii) the effect of BC on earthworms (*Eisenia fetida* Sav.). The vermicompost was co-produced over 5 months (n = 4 per treatment) using (i) grape bagasse as the substrate, (ii) earthworms (*Eisenia fetida* Sav.), and (iii) three BCs (eucalyptus sawdust BC, pig manure BC, and carbonaceous material from poultry litter CM) at 2% (*w*/*w*). A control without BC was included. The chemical, microbiological (activity and respiration), enzymatic properties, and enzymatic indices were characterized. After the incubation period, vermicompost yield increased with the application of the three BCs (25% on average). The number of adult earthworms was not affected by any of the BCs. Compared to treatments without BC, those with pig manure BC and eucalyptus BC resulted in maintained or significantly decreased enzymatic activity, indicating that the vermicompost was at an advanced stage of maturity. Eucalyptus BC significantly enriched the C content of the vermicompost by 4.3%, maintaining respiration rates at 18% lower than the treatment without BC. Additionally, pig manure BC generated the lowest respiration rate in the vermicompost (20% lower). We conclude that BC has a positive influence on the vermicompost process, stabilizing organic matter (especially pig manure BC) and improving the potential of vermicompost to store C (when high-C-content BCs are applied).

Keywords: waste management; earthworms; grape bagasse; biochar

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1. Introduction

The utilization of earthworms to manage organic waste, known as vermicomposting, has gained attention due to its potential to produce high-quality products such as vermicompost, which can improve soil fertility and stimulate plant growth [1,2]. This is due to the production of a rich source of enzymes with the capacity to detoxify soil contaminated with pesticides [3]. One organic material that has been studied for vermicomposting is grape bagasse, the fibrous residue of winemaking. Recent research has shown that vermicomposting grape bagasse leads to significant changes in the composition of bacterial communities, indicating that there is microbial activity during this process [2]. The importance of changes in the activity and quantity of microbial communities is associated

with the phase of compost maturation, which is linked to the stabilization of organic matter [2]. Furthermore, it has been found that vermicomposting of grape bagasse produces organic biofertilizers of high quality, enriched with nutrients and microbial activity [3]. The vermicomposting of grape marc, which includes bagasse, has been proven to enhance soil fertility and promote plant growth, making it a sustainable method for managing organic waste in the wine industry [4,5]. Vermicompost enhances soil biodiversity by promoting beneficial microbes, which in turn stimulate plant growth through the production of plant-growth-regulating hormones and enzymes [3,6]. Its application has been demonstrated to improve soil characteristics such as pH, nutrient content, humification ratios, and electrical conductivity [6]. Moreover, vermicompost derived from grape bagasse has been found to possess adsorption capabilities, making it effective in removing pharmacological substances from water solutions [7]. The use of grape bagasse for vermicomposting offers not only a sustainable waste management solution for the wine industry but also a pathway for producing a valuable organic fertilizer to enhance soil fertility and plant growth [8]. Overall, the vermicomposting of grape bagasse presents a promising approach for producing high-quality vermicompost and for the sustainable management of organic waste.

Biochar is a carbonaceous material resulting from the pyrolysis of carbon-rich biomass, including forest or animal wastes [9,10]. When used as a soil amendment, it has multiple co-benefits for environmental and agronomic performance [10,11]. Biochar is characterized by its high C stability and abundant surface functional groups, e.g., carboxylic, phenolic, hydroxyl, carbonyl, and quinone groups [12]. It can alter the biological activity in soils and other substrates [13–15] and, owing to its absorption ability, could contribute to the remediation of organic compounds in soils [13]. Previous studies have investigated the interactions between biochar and earthworms [13]. Usually, biochar has a neutral or positive effect on earthworms in soil environments [16], however, ref. [17] reported high earthworm mortality following the application of poultry litter biochar.

In combination, biochar and earthworms can modify the soil microbial community, changing the abundance and activity of microorganisms in addition to enzymatic activity [13–15,18]. In fact, [13] found that the combined use of biochar and earthworms can potentially increase productivity in acidic soils and be part of a sustainable soil management strategy. Potential trophic effects of biochar on earthworms are possible, and some studies on biochar ingestion have shown a preference for soil–biochar mixtures [19,20]. However, there remain gaps in our understanding of the effects of the interactions between biochar and earthworms on biochemical activity.

Based on this background, there is scientific interest in investigating the impact of biochar on earthworm growth, reproduction, and the quality of vermicompost [14]. For instance, research has demonstrated that the introduction of biochar to precomposted sewage sludge mixtures can have a positive effect on the growth and reproduction of *Eisenia fetida* Sav. during laboratory vermicomposting [21]. Moreover, other studies have explored the biological and chemical reactivity of vermicompost and compost mixtures containing biochar, as well as the potential of biochar to immobilize heavy metals during the vermicomposting process [22]. The negative impacts of high concentrations of biochar on earthworms (decrease in biomass and density of earthworms) have been reported at doses of 20% and 25% [23]. Conversely, a lower dose of 5% biochar was found to be sufficient for enhancing the quality of vermicompost [23].

Biochar addition in vermicomposting has been found to have varying effects on enzymatic activity [6,23,24]. For instance, the activity of alkaline phosphatase, aminopeptidase, and N-acetylglucosaminidase increases with biochar application [24]. When evaluating the optimization of biochar addition in vermicomposting, ref. [6,23] found that higher doses of biochar are associated with increased enzymatic activity, indicating enhanced microbial activity and nutrient cycling. However, other studies have reported that vermicompost reduces the activity of alkaline phosphatase [6]. These findings suggest that the effect of biochar on enzymatic activity in vermicomposting can vary depending on the specific conditions, phase, and parameters of the system. Further research is needed to

fully understand the changes produced by the addition of biochar to the vermicomposting process. We hypothesize that the application of biochar improves vermicompost productivity and quality without adversely affecting earthworms. Therefore, this research aimed to (1) assess how the addition of different types of biochar affects the yield of vermicompost and its biochemical, chemical, and biological properties, and (2) evaluate the effect of BC on earthworms (*E. fetida*) based on a comprehensive understanding of the interactions between biochar and earthworms during vermicomposting. The findings represent applied knowledge related to the management of agricultural waste.

2. Materials and Methods

2.1. Production of Biochar

For the study, waste materials from poultry and pig livestock farms were utilized, as well as eucalyptus wood residues sourced from producers in central-southern Chile. The biochars were produced following the method outlined in [25]. The waste materials were first dried to a humidity level below 20%, and the biochars were then produced by slow pyrolysis (maximum temperature 550 °C) under a nitrogen atmosphere (flux 1 L min^{-1}) at a heating rate of 20 °C min^{-1} up to the target temperature (550 °C) using a pilot kiln at laboratory scale. The total residence time was 2 h. The gases produced during the pyrolysis process were condensed and recovered as a liquid fraction (bio-oil). This process resulted in the production of three types of biochar: eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM). It is worth noting that CM has a relatively low organic carbon content (OC = 5.64%) and does not meet the criteria for biochar as defined by the European Biochar Certificate and the International Biochar Initiative (IBI) guidelines (OC values above 10%). The characteristics of these materials have been previously examined by [25] (Table 1).

Table 1. Chemical characterization of materials.

Parameters	Unit	[1] BC_1	[1] BC_2	[1] CM	[1] B
pH	–	5.73	8.2	10.24	6.78
CE (1:5)	dS m^{-1}	–	–	–	2.14
MO	%	–	–	–	95.45
TN	g kg^{-1}	2.8	33.9	8.4	18.4
TC	g kg^{-1}	841.5	418.8	116.1	553.7
[2] Cno_ox	g kg^{-1}	760.2	124.4	71.1	-
C:N	–	300.5	12.4	13.8	30.09
$N\text{-}NO_3^-$	mg kg^{-1}	380	7860	9400	25.5
$N\text{-}NH_4^+$	mg kg^{-1}	0.55	9.58	5.79	79.8
Av. K	g kg^{-1}	0.32	16.15	14.78	11.29
Av. P	g kg^{-2}	1.58	13.17	22.19	2.71
Av. Mg	g kg^{-3}	0.06	12.85	12.32	1.09
Av. Ca	g kg^{-4}	0.71	30.57	223.8	4.93
$CaCO_{3\text{-eq}}$	%	0.39	2.45	12.69	–
S	mg kg^{-1}	<d.l	260	230	–
Fe	mg kg^{-1}	–	–	–	295
Mn	mg kg^{-1}	–	–	–	17
Zn	mg kg^{-1}	–	–	–	7
Cu	mg kg^{-1}	–	–	–	16
B	mg kg^{-1}	–	–	–	20
WB Moisture	%	–	–	–	42.4

[1] Eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM); vermicompost from bagasse (B); [2] Cno_ox corresponds to the sum of alkyl and pyrogenic C that is not oxidized after mixing with a concentrated dichromate solution, and it is contained only in pyrogenic materials such as biochars [12].

2.2. Experimental Setup

The research was carried out during the winter–spring seasons (July to November 2020) in the Ñuble region (36°54′35.8″ S; 72°24′40.5″ W) situated in central-southern Chile, utilizing grape bagasse of the Pais variety (*Vitis vinifera* L.) sourced from vineyards in the Itata Valley. Prior to the beginning of the experiments, this material underwent a composting process in the field for a duration of 7 weeks to eliminate an overabundance of phytotoxic compounds. The process of vermicomposting was carried out for 5 months in a room where environmental conditions were controlled, including darkness and the room temperature ranging between 10 and 26 °C, which is suitable for vermicomposting development [26]. During winter, the environmental temperature outside the room dropped to less than 5 °C, reaching −1.8 °C, a condition that could potentially hinder the performance of *E. fetida*. As a result, we used a heater to warm the incubation room and ensure the earthworms were not negatively affected. The incubation process was realized using plastic containers with a capacity of up to 6 L that were pierced at the bottom to hinder the build-up of leachate. Inside each container, 2 kg of grape bagasse was mixed with each of the obtained biochars and carbonaceous materials to a proportion of 2% (*w*/*w*) substrate, maintaining its humidity at approximately 80%. In addition, 100 *E. fetida* earthworm species, obtained from commercial gardening vermicomposting, were incorporated. The earthworms were selected evenly from both the adult and juvenile populations to ensure species survival and reproduction. The overall biomass of the earthworms was carefully tracked and standardized, with four repetitions of each treatment setup. After the incubation period, the earthworms were manually separated, and the content of each container was then sieved (2 mm) to obtain the stabilized products. The material was stored at 4 °C until analysis.

2.3. Treatments

Five treatments were established in a completely randomized design with four replicates each, termed T0: B (control, bagasse); T1: B + E (vermicompost); T2: B + E + BC_1; T3: B + E + BC_2; T4: B + E + CM. B represents bagasse, E represents earthworms, BC denotes biochar of each type, and CM denotes carbonaceous material (Table 1).

2.4. Chemical Analysis

Total C and N content was determined via dry combustion using an elemental analyzer (Truspec CN, LECO, St. Joseph, MI, USA). The pH, organic matter, and contents of N-NO_3^-, N-NH_4^+, available P, K, Ca, and Mg, as well as micronutrients, such as B, Cu, and Mn, were obtained from composite samples of each treatment following procedures corresponding to the standard protocols for compost analysis [27].

2.5. Preparation of Samples for Enzymatic Assays

The procedures laid out by [28] were followed to measure enzymatic activities in vermicompost–water extracts, maintaining a ratio of 1:50 (*w*/*v*). The enzymatic assays for acid phosphatase, β-glucosidase, urease, and protease required extended reaction periods of 4 h. As a result, sodium azide (NaN_3), at a final concentration of 1 mM, was also introduced to the mixtures to restrain microbial proliferation, as suggested by [28]. To avoid interference with the formation of the Fast Red–naphthol complex, NaN_3 was later incorporated into the carboxylesterase (CbE) determinations.

2.6. Enzymatic Activity

A group of enzymes linked to carbon cycles (carboxylesterase and β-glucosidase), nitrogen (protease and urease), and phosphorus (acid phosphatase) was selected for our research. The functioning of dehydrogenase and catalase was evaluated as a measure of soil microbial activity, as outlined by [29,30]. The method used was proposed in [29] to determine dehydrogenase activity, while catalase activity was determined following the method described in [30]. The activity of carboxylesterase was evaluated following

the approach suggested by [31]. Acid phosphatase and β-glucosidase activities were measured using the method in [32]. The protease activity was measured using the method suggested by [33]. However, the reactions were performed in 1.5 mL microcentrifuge tubes to minimize reagent use. The method described by [33] was used to determine urease activity.

2.7. Microbial Activity

The assessment of microbial activity was conducted via the hydrolysis of fluorescein diacetate (FDA), resulting in the fluorescence of FDA hydrolysates [34]. This technique is used to measure the active microbial activity of a substrate. Absorbance was measured using a UV/VIS spectrophotometer (Thermo Spectronic model Genesys TM 5, Vernon Hills, IL, USA) at a wavelength of 490 ηm.

2.8. Microbial Respiration

Microbial respiration was analyzed by determining the accumulated specific respiration through measurements of carbon dioxide (CO_2). These measurements were obtained from products formed during vermicomposting using infrared spectroscopy with a CO_2 gas analyzer (Li-820, LI-COR Bioscience, Shelton, CT, USA) on incubated substrates, and by monitoring CO_2 evolution at 3-, 5-, and 7-day intervals. For all of the different treatments, the incubations were conducted at 22 °C [35], where 15 g of each sample was placed in 50 mL Falcon tubes with rubber septum caps for CO_2 sampling. The data obtained at each time interval were analyzed using linear interpolation according to [36].

2.9. Enzymatic Index

Three numerical indices were used to assess the impact of treatments on the enzymatic activities of vermicompost: the GMean index [37], the treated soil quality index (T-SQI; [38]), and the integrated biological response index version 2 (IBRv2; [39]). The calculations for each index are detailed below.

The GMean index was calculated according to Equation (1) [37]:

$$\text{GMean} = \left(\prod_{i=1}^{n} y_i\right)^{1/n} \quad (1)$$

the T-SQI was evaluated according to Equation (2) (T-SQI; [38]):

$$T - SQI = 10^{\log m + \frac{\sum_{i=1}^{n} (\log n_i + \log m) - \sum_{i=1}^{n} |\log n_i - \log \bar{n}|}{n}} \quad (2)$$

where m is the control vermicompost (T1; average enzymatic activity, taken as the reference point of 100%) and n is the average of each enzymatic activity in the treated vermicompost, expressed as a percentage relative to the reference control.

Finally, the IBRv2 values are obtained as the sum of deviations between the reference (vermicompost, T1) and the vermicompost with the treatments incorporated. It is calculated using Equation (3) (IBRv2; [39]):

$$\text{IBRv2} = \sum_{i=1}^{n} |A_i| \quad (3)$$

the T-SQI and IBRv2 values were illustrated in sunburst charts to visually evaluate the enzymatic responses of each treatment compared to the reference.

2.10. Statistical Analysis

The variables were statistically analyzed using analysis of variance (ANOVA) after checking the assumptions of homogeneity of variance (Levene's test) and normality of residuals (Shapiro–Wilk test). For CO_2 emissions, data were logarithmically transformed as they did not meet the assumptions of homoscedasticity and normality. For enzymatic analyses, the Kruskal–Wallis test followed by the pairwise Wilcoxon test was applied to

compare enzymatic activities. Variables showing significant differences were subjected to Fisher's LSD test. The statistical analyses were conducted using InfoStat software version 2020 [40].

3. Results

The number of adult earthworms (as shown in Table 2) obtained at the end of the period did not vary across all treatments, however, the number of juvenile individuals was not countable. The yields of vermicompost showed highly significant differences among the treatments (p-value ≤ 0.0001), with the animal waste treatments (T3 and T4) exhibiting higher values (Table 2).

Table 2. Final adult earthworm count and amendment yield (g) in response to applied treatments.

Treatments	Adult Earthworms	Yield VC (g)
T_0: B	-	62.35 ± 2.61 c
T_1: B + E	97 ± 2.65	237.43 ± 30.80 b
T_2: B + E + BC_1	85 ± 14.97	266.75 ± 38.69 b
T_3: B + E + BC_2	90 ± 5.19	306.38 ± 16.81 a
T_4: B + E + CM	94 ± 4.92	311.38 ± 15.37 a
p-value	0.30	0.0001
CV %	9.2	10.29

Different lowercase letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$). Mean ± standard deviation (n = 4). Vermicompost (VC), bagasse (B), earthworms (E), eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM).

The C/N ratio showed highly significant differences between the treatments (p-value < 0.0001), with vermicompost (T_1), vermicompost + pig manure biochar (T_3), and vermicompost + carbonaceous material from poultry litter (T_4) having the lowest values, while the highest C value was observed for the treatment in which eucalyptus BC was added (Table 3). The other parameters are reported as descriptive data of the final products.

Table 3. Chemical characterization of products at final incubation time in response to applied treatments.

Parameters	Units	T_0: B	T_1: B + E	T_2: B + E + BC_1	T_3: B + E + BC_2	T_4: B + E + CM
pH (1:5)	--	5.51	5.03	4.92	5.76	6.61
OM	%	90.66	84.99	85.98	78.37	65.62
Total N	%	3.56 ± 0.15 ab	3.74 ± 0.18 a	3.33 ± 0.06 b	3.66 ± 0.12 a	2.94 ± 0.28 c
Total C	%	46.60 ± 0.18 a	43.65 ± 1.91 b	48.40 ± 3.10 a	42.73 ± 1.23 b	35.0 ± 1.69 c
C/N Ratio	--	13.10 ± 0.53 b	11.69 ± 0.18 c	14.55 ± 1.12 a	11.69 ± 0.11 c	11.97 ± 0.58 c
$N\text{-}NO_3$	mg kg^{-1}	291.90	1325.6	153.6	555.80	734.1
$N\text{-}NH_4$	mg kg^{-1}	62.70	85.10	72.70	60.20	50.30
Av. N	mg kg^{-1}	354.6	1410.7	226.3	616	784.4
Av. P	g kg^{-1}	3.36	3.62	2.83	11.42	8.33
Av. K	g kg^{-1}	26.64	22.08	18.43	18.68	18.01
Av. Ca	g kg^{-1}	7.29	7.72	6.51	13.44	74.07
Av. Mg	g kg^{-1}	2.41	2.29	1.93	4.70	3.38
Mn	mg kg^{-1}	42	105	108	273	22
Cu	mg kg^{-1}	52	48	39	88	53
B	mg kg^{-1}	30	41	32	48	37

Different lowercase letters indicate significant differences according to Fisher's LSD test ($p \leq 0.05$). Mean ± standard deviation (n = 4). Bagasse (B), earthworms (E), eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM).

Highly significant differences ($p < 0.0001$; CV = 26.6%) in microbial respiration were identified between the applied treatments (Figure 1), with the highest cumulative respiration observed in T_0 (bagasse) and T_4 (vermicompost with CM), while vermicompost with pig manure BC and eucalyptus BC resulted in reduced respiration rates.

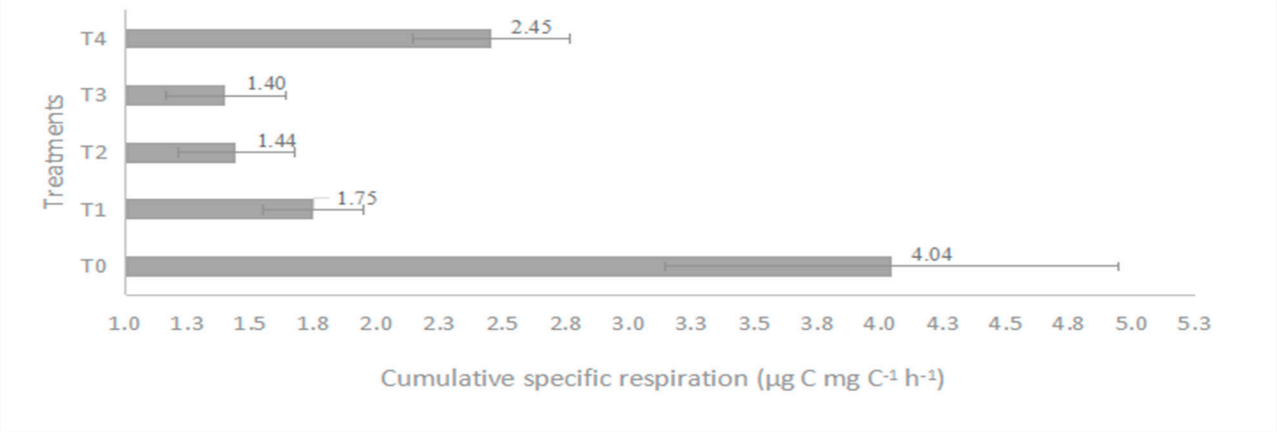

Figure 1. Cumulative specific respiration (µg C mg C^{-1} h^{-1}) during seven days of incubation for each treatment. T_0: B (bagasse); T_1: B + E (vermicompost); T_2: B + E + BC_1; T3: B + E + BC_2; T4: B + E + CM. Bagasse (B), earthworms (E), eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM).

Table 4 shows the enzymatic activity of the vermicomposts at the end of the incubation time. Significant differences between treatments were only obtained for FDA activity. The lowest values of FDA were observed in the treatment with pig manure BC (T_3).

Table 4. Enzymatic activity of vermicomposts after 5 months of incubation.

Enzymes	T_1: B + E	T_2: B + E + BC_1	T_3: B + E + BC_2	T_4: B + E + CM	*p*-Value
Des (µmol INFT h^{-1} g^{-1})	1.96 ± 0.76	2.41 ± 0.18	1.47 ± 0.34	2.68 ± 0.70	0.11
Cat (mmol H_2O_2 h^{-1} g^{-1})	91.13 ± 7.61	73.84 ± 8.51	79.35 ± 3.7	93.80 ± 15.66	0.15
Est 1-NB (µmol h^{-1} g^{-1})	374.55 ± 245.6	331.19 ± 204.3	192.05 ± 69.9	347.52 ± 224.9	0.41
Est 4-NPB (µmol h^{-1} g^{-1})	2298.76 ± 900.8	1463.2 ± 436.8	992.97 ± 657.1	1442.99 ± 576.5	0.13
Ac. Phos (µmol h^{-1} g^{-1})	38.37 ± 12.92	28.87 ± 8.73	34.42 ± 10	35.34 ± 16.84	0.78
Glu (µmol h^{-1} g^{-1})	8.29 ± 3.15	8.96 ± 3.14	8.75 ± 4.26	15.73 ± 5.04	0.11
Prot (mg Tyr eq $h^{-1}g^{-1}$)	13.53 ± 2.22	12.49 ± 1.15	11.85 ± 5.62	13.57 ± 1.28	0.46
Ure (µg NH_4^+ h^{-1} g^{-1})	213.69 ± 39.27	188.71 ± 21.47	144.60 ± 45.96	169.91 ± 40.16	0.29
FDA (µg F g^{-1})	13.62 ± 1.84 a	16.22 ± 5.59 a	5.53 ± 0.43 b	7.40 ± 0.62 ab	0.005 **

Dehydrogenase (Des), catalase (Cat), esterase 1-NB (Est-1NB), esterase 4-NPB (Est-4NPB), acid phosphatase (Ac. Phos), β-glucosidase (Glu), protease (Prot), urease (Ure), and FDA. T0: B (bagasse); T_1: B + E (vermicompost); T_2: B + E + BC_1; T_3: B + E + BC_2; T4: B + E + CM. Bagasse (B), earthworm (E), eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM). Different lowercase letters indicate significant differences according to the Kruskal–Wallis test (*p*-value ≤ 0.05) or highly significant ** (*p*-value ≤ 0.01). Mean ± standard deviation (n = 4).

The overall enzymatic activity of the vermicompost is shown in terms of the GMean, T-SQI, and IBRv2 indices (Table 5). The lowest activity was found in the T3 (vermicompost + pig manure biochar) treatment in the T-SQI and GMean indices, while no significant difference was observed in the IBRv2 index. The distribution of the (a) T-SQI and (b) IBRv2 indices is shown in Figure 2.

Table 5. Global enzymatic activity reflected by T-SQI, IBRv2, and GMean index scores for vermicompost at the final incubation time.

Treatments *	T-SQI	IBRv2	GMean
T_1: B + E	-	-	48.58 ± 10.3 a
T_2: B + E + BC_1	64.58 ± 10.7 ab	2.49 ± 1.03	43.33 ± 2.9 a
T_3: B + E + BC_2	45.95 ± 14.6 b	8.93 ± 7.36	32.93 ± 10.2 b
T_4: B + E + CM	69.04 ± 9.6 a	1.16 ± 2.1	45.44 ± 5.6 a
p-value	0.05 *	0.07	0.04 *

* T_1: B + E (vermicompost); T_2: B + E + BC_1; T_3: B + E + BC_2; T4: B + E + CM. Bagasse (B), earthworms (E), eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM). Different lowercase letters indicate significant differences according to the Fisher LSD test (*p*-value ≤ 0.05). Mean ± standard deviation (n = 4).

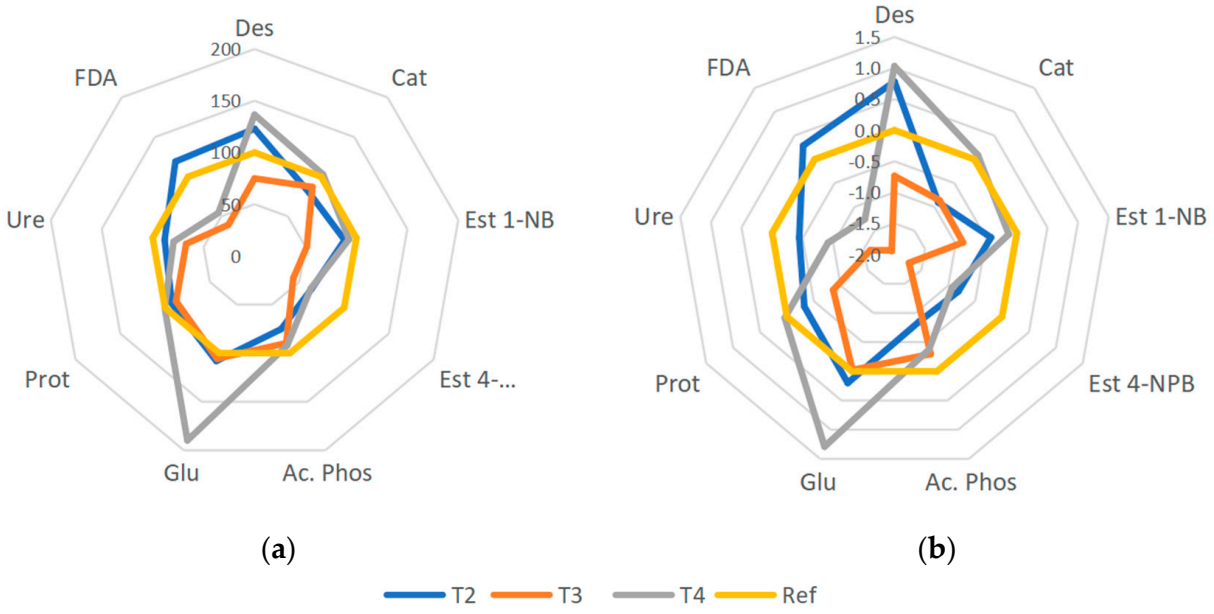

Figure 2. Global enzymatic response for vermicompost with added biochars through distribution of (**a**) T-SQI and (**b**) IBRv2 scores. Dehydrogenase (Des), catalase (Cat), esterase 1-NB (Est-1NB), esterase 4-NPB (Est-4NPB), acid phosphatase (Ac. Phos), β-glucosidase (Glu), protease (Prot), urease (Ure), and FDA. T_1: B + E (vermicompost); T_2: B + E + BC_1; T_3: B + E + BC_2; T4: B + E + CM. Bagasse (B), earthworms (E), eucalyptus sawdust biochar (BC_1), pig manure biochar (BC_2), and carbonaceous material from poultry litter (CM).

4. Discussion

4.1. E. fetida Population and Yield and Chemical Properties of Vermicompost

There was no reduction in the adult earthworm population after five months of experimentation in the presence of biochar compared to the vermicompost without biochar or carbonaceous material (T1) (see Table 2), suggesting that these materials are not toxic to earthworms. In contrast, ref. [41] highlighted the toxicity of biochar poultry litter on *E. fetida*, attributed to the harmful effects of ammonia and increased pH levels in poultry litter biochar treatments. In their study, there was a high rate of BC application (22.5, 45, 67.5, and 90 Mg ha^{-1}), whereas we utilized a 2% (*w*/*w*) ratio with respect to the bagasse substrate. Consequently, although the pH levels in treatments T3 (vermicompost + pig manure biochar) and T4 (vermicompost + carbonaceous material from poultry litter) were higher than those for bagasse (T0) and vermicompost (T_1) (Table 3), they did not rise above pH 7 at the end of the experiment, so the conditions should not have been lethal to earthworms [42]. Other research has indicated an uptick in the reproduction rate toward

the end of the experiment [43,44]. However, factors such as the duration of our study (five months) and low winter temperatures (10 °C) could have led to a reduction in the reproduction rate. This is because earthworms reduce their activity in cooler conditions, with the optimum temperature for *E. fetida* being 25 °C [45].

In line with our findings, the authors of [44] found that the use of paper mill sludge in vermicomposting resulted in a higher yield due to its contribution to the organic C content, which in turn affects the reproduction of *E. fetida* and the vermicompost yield [44]. In this study, all treatments boosted the yield of vermicompost (in the order CM = BC_2 > BC_1). Furthermore, the elevated values of C in the vermicompost that included eucalyptus BC are linked to the high Cno_oxi content (Table 1). Comparable findings in soil were reported by [25], who observed that the material rich in carbon from poultry litter led to reduced C content in soil, while eucalyptus BC increased soil C under field conditions.

The low C/N ratio observed in the treatments vermicompost (T_1), vermicompost + pig manure biochar (T_3), and vermicompost + carbonaceous material from poultry litter (T_4) can be attributed to the limited initial ratio of grape bagasse [3]. A C/N ratio fluctuating between 20 and 28 has been identified as the reference range for chemical parameters in vermicomposting livestock waste, as reported by [46]. This determinant is crucial in biological processes since it is a major factor in the maturity of the amendment. This maturity is assessed by the reduction of organic carbon, which results from the release of CO_2 facilitated by the respiration of organisms engaged in the process [44,47]. In the case of vermicompost with eucalyptus sawdust biochar (T_2), the C/N ratio remained unchanged, as previously observed, due to the high Cno_oxi concentration in the eucalyptus BC. Table 3 shows that vermicomposts with animal waste (T3 and T4) have the highest P content. Animal manure BCs are usually rich in ash [48] and provide high contents of available nutrients [48–50]. Our previous results from volcanic soils show that pig and poultry manure BCs increase the yields of clover [51] and sorghum [25] due to their effect on soil pH and soil available P. The results obtained here indicate that the addition of C-rich BC enriches the stable C content of vermicompost, while ash-rich BC increases the pH and available P of vermicompost, which could be strategically leveraged for the creation of amendments that contribute to increasing OC in agricultural soils and/or associated crop yield.

4.2. Microbial Respiration

The reduced rate of microbial respiration in vermicompost treatments, particularly those featuring pig manure and eucalyptus BCs, points to the final vermicompost being in an advanced stage of maturation when compared with bagasse T0. The high emission of CO_2 is associated with the initial stages of vermicomposting, which typically has substantial labile C. In the vermicomposting of grape, peak CO_2 production was observed after 30 days due to the heightened activity of worms and microorganisms [48]. This figure, however, experiences a significant drop during the stabilization phase [43,46]. In this case, following a five-month period, we found a mature vermicompost with diminished total C and CO_2 emissions compared to the grape bagasse (T0). Based on our results, future studies on vermicomposting where BC is applied could focus on determining from which stage the respiration rates begin to decrease. This would allow for decreasing the time and costs associated with the production of stable vermicompost.

In this research, both pig biochar and eucalyptus biochar were found to reduce CO_2 emissions from the vermicompost compared to treatment T1 (vermicompost without BC). Typically, BCs have low mineralizable C [52] and a high fixed C index [53]. Thus, even though BCs are not entirely resistant to biological degradation, the CO_2-C released from biochar constitutes only a minor fraction of the biochar C and does not threaten the capacity of increased soil C stocks [54,55]. In this context, it was noticed that eucalyptus BC reduced the respiration rate while augmenting the total C content of the vermicompost (T_2—vermicompost + eucalyptus sawdust biochar). Contrarily, the increased respiration in vermicompost + carbonaceous material from poultry litter (T_4) when compared to other

vermicomposts is linked to the high concentration of $CaCO_3$ in the poultry litter CM (Table 1). Table 3 shows that (vermicompost + carbonaceous material from poultry litter had a high Ca content of 74.07 g Ca kg^{-1} even after 5 months of CM application. In their study, the authors of [25] determined that chicken manure CM can increase cumulative CO_2 emissions due to the discharge of carbonates, a phenomenon that tends to be more significant in acidic substrates [56].

4.3. Enzymatic Activity and Global Index

Our findings indicate that the BC treatments have either equivalent or lower enzyme activity than the vermicompost control (refer to Table 4). High enzymatic activity is common at the beginning of the vermicomposting process [43] due to the action of microbes in the presence of earthworms [8]. Consequently, an elevated level of enzyme activity could suggest that the vermicompost is not yet fully mature. Biochars of wheat and maize straw are recognized as rich carbon additives that facilitate carbohydrate metabolism [57] by breaking down cellulose and hemicellulose. However, as maturation progresses, these processes decrease in intensity due to the reduced availability of new material [8]. Therefore, it was expected that when assessed with these indicators, the treatments would score lower than the reference, with the exception of T_4 (vermicompost + carbonaceous material from poultry litter). The T_4 treatment resulted in the increased activity of dehydrogenase and β-glucosidase, enzymes that are linked to microbial activity and the carbon cycle. This is in alignment with its values of microbial respiration (Figure 1) and carbonate content (Table 1). On the other hand, T_3 (vermicompost + pig manure biochar) appeared to be in a more advanced stage of maturation.

The T-SQI index is recommended for the assessment of soil quality after treatment with amendments [38] and the detection of pesticides in farming soils [57]. Conversely, the IBRv2 index is utilized for gauging the health of animals living in polluted surroundings [39]. In our research, the T-SQI index was found to be more finely tuned to the remaining evaluated parameters (such as respiration and enzymes). For example, the lowest T-SQI index value was observed in T_3 (vermicompost + pig manure biochar), where the FDA and respiration rates were also the lowest. Nonetheless, the linear correlation between the two indices (T-SQI and IBRv2) indicates that they were well matched ($r = 0.87$; Figure S1). This suggests that both methods can be helpful in showing the overall enzymatic activity in vermicompost treated with biochar, as an indicator of the final quality (or maturity) of the obtained compost. However, a limitation of our study is that enzyme activity was only measured at the end of the trial. It is suggested that enzyme activity should be evaluated at different vermicomposting stages in future research.

Figure 2 shows elevated dehydrogenase and β-glucosidase enzymes in T_4 (vermicompost + carbonaceous material from poultry litter), which was above the reference (vermicompost). The high respiration rate observed for this treatment is linked to the application of carbonate-rich CM. However, the activity of all enzymes in T_3 (vermicompost + pig manure biochar) was below the reference. These findings suggest that, of the treatments, T_3 treatment contains vermicompost of the most advanced level of maturity, which is due to the application of pig BC. We believe that this is a notable finding, given that the maturity of vermicompost is related to the stability of the amendment and its fertilizer value.

5. Conclusions

Our research findings demonstrate that the incorporation of a 2% biochar quantity, sourced from forestry and livestock systems, can effectively enhance the vermicomposting process of grape bagasse, leading to higher final yields without harming earthworms. The biochars facilitated the stabilization of organic substances, as indicated by the lowered CO_2 emissions, excluding the case of poultry litter CM. The reduced enzymatic activities are indicative of the maturation stage of the vermicompost. It is important to highlight that the optimal selection of biochar for this process is influenced by the initial raw material

employed and the final intended use of the vermicompost. Our study demonstrates that biochar sourced from pig manure notably improves the stabilization of organic substances, thus offering significant potential for further exploration into the interactions between vermicompost and biochar.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030615/s1, Figure S1: Relationship between T-SQI and IBRv2 scores in response to enzymatic activities of vermicompost with added biochar.

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