

Article

Effect of Biochar as an Additive in Co-Composting: Impacts on Physicochemical Properties, Enzyme Activity, and Substrate Quality

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Abstract

The growing demand for sustainable alternatives to non-renewable growing media components, such as sphagnum peat, has driven interest in compost–biochar combinations as high-quality horticultural substrates. Nevertheless, the effects of incorporating biochar at the onset of co-composting on process dynamics and the quality of the resulting end-products remain poorly understood and insufficiently characterized. This study evaluated the influence of hardwood residue-derived biochar as an additive on the co-composting of corn stover and swine manure under greenhouse conditions over 94 days. Three treatments were established: a Control (C; without biochar), CB1 (10% *v/v* biochar), and CB2 (20% *v/v* biochar), each with three replicates. Physicochemical properties (pH, electrical conductivity, water holding capacity, bulk density, particle size distribution, total organic carbon), enzyme activities (FDA hydrolysis, dehydrogenase, urease, arginine ammonification, acid phosphatase, arylsulfatase), compost stability (Solvita[®] test), and phytotoxicity (germination index) were monitored throughout the process and at process completion. Results showed that biochar addition significantly influenced physicochemical and biological parameters in a dose-dependent manner. The CB2 treatment reduced electrical conductivity by 46% relative to the Control (1.87 vs. 3.45 mS/cm) and increased water-holding capacity by 32% (479 vs. 364%), while all treatments met the Chilean composting standard NCh 2880. Enzyme activities were generally higher in the Control and CB1 treatments, indicating that high biochar rates may limit microbial activity due to recalcitrant carbon inputs, yet a 20% biochar addition improved compost maturity and reduced phytotoxicity. These findings indicate that, within the tested range (10–20% *v/v*), biochar incorporation appears to enhance the quality, supporting its potential as a sustainable component in growing media formulations as an alternative to peat.

Keywords: organic amendment; biological activity; compost; physicochemical properties



Academic Editor: Juan Antonio López González

Received: 23 April 2026

Revised: 24 May 2026

Accepted: 27 May 2026

Published: 30 June 2026

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1. Introduction

The growing global population and the resulting demand for food production have led to significant environmental impacts, particularly through the generation of agricultural and agro-industrial residues. This situation underscores the urgent need to develop sustainable technologies for the proper management of these organic materials to mitigate their negative environmental effects [1–3]. It is estimated that the global annual production of crop residues such as rice, corn, and wheat reaches approximately 45 Tg [4–7]. Without appropriate management, these residues can pose a serious environmental threat, as evidenced by practices such as stubble burning [8]. However, these carbon-rich materials also offer opportunities for transformation into value-added products, thereby reducing environmental pressure and preventing waste accumulation to promote sustainable systems.

Agricultural residues are highly valuable due to their high organic matter (OM) and mineral content, making them suitable raw materials for the development of agricultural bioproducts [8–10]. When incorporated into soils, these materials can improve biological, chemical, and physical soil properties and enhance crop yields [11,12]. Composting, a process involving the decomposition, transformation, and stabilization of OM, can reduce the volume of raw materials by up to 50%, resulting in a stable product that functions as a biofertilizer [13–15].

Various organic feedstocks have been evaluated for compost production and as components of soilless growing media [16–19]. However, compost and the process of composting can present limitations and issues such as rapid mineralization, high decomposition rates, emissions of gases, and elevated salinity, which may hinder its use in growing media formulations [19]. To address these challenges, the incorporation of biochar, a carbon-rich material produced by pyrolysis of organic residues, has gained increasing attention. Biochar shares many physicochemical characteristics with peat (*Sphagnum* sp.), including high porosity, low bulk density, and elevated cation exchange capacity (CEC), making it a promising peat alternative [18,19]. It is produced through pyrolysis under oxygen-limited conditions at temperatures ranging from 300 to 800 °C, during which depolymerization, oxidation, dehydration, and decarboxylation reactions occur [2,20–23]. Biochar production can also contribute to climate change mitigation by enhancing carbon sequestration and reducing CO₂ emissions and increasing the mean residence time of amendments [24,25]. Despite certain limitations, such as relatively low nutrient content and potential phytotoxicity at high concentrations, positive results have been reported when biochar is used at rates below 50% in growing media formulations [19,26].

In recent years, the combination of compost and biochar has emerged as a promising strategy for producing high-quality growing media. While compost provides nutrients and microbial activity, biochar contributes with its physical structure and chemical stability, resulting in a synergistic effect that enhances the overall quality of the mixture. This combination can improve key substrate properties such as porosity, water-holding capacity, aeration, and CEC, while simultaneously reducing risks such as salinity, rapid mineralization, and the presence of phytotoxic compounds [18,19]. Moreover, studies have reported that biochar can reduce the total and available concentrations of heavy metals in composting, stabilize organic matter, and promote beneficial microbial activity [17,27,28]. Given the environmental concerns associated with peat extraction, the compost–biochar combination presents a sustainable and effective alternative for soilless cultivation systems. Despite the growing body of literature on biochar–compost interactions, critical knowledge gaps remain. Most studies have incorporated biochar after the composting process rather than from its onset, limiting our understanding of how biochar shapes microbial dynamics throughout composting.

Furthermore, comprehensive enzymatic profiling covering the cycles of carbon, nitrogen, phosphorus, and sulfur simultaneously has rarely been reported for co-composting systems despite their sensitivity as indicators in soil and diverse processes [29]. Finally, benchmarking the final product against standards for soilless growing media, particularly as a potential peat substitute, has received limited attention in experimental studies using composted agricultural residue feedstocks. This study addresses these gaps by evaluating the effects of two rates of hardwood-derived biochar incorporated from the beginning of co-composting on the physicochemical and biological quality of the composting process and the end-product, with a focus on its potential as a sustainable component in growing media formulations. We hypothesize that integrating biochar into the composting of agricultural residues not only enhances composting efficiency but also improves the quality of the end-product, making it a viable alternative in growing media formulations. To our knowledge, this is the first study to simultaneously (i) incorporate hardwood-derived biochar at the onset (rather than after completion) of a co-composting process using corn stover and swine manure as feedstocks; (ii) profile a comprehensive suite of six enzyme activities linked to C, N, P, and S cycling as real-time indicators of microbial functional diversity throughout the co-composting process; and (iii) systematically benchmark the resulting compost–biochar end-products against the Chilean growing media standard (NCh 2880) as potential sphagnum peat substitutes. This combination of process monitoring, multi-enzyme profiling, and standardized product quality assessment represents a novel methodological approach in the context of agricultural residue co-composting research. Given this context, the present study aims to (i) evaluate the influence of two rates of hardwood-derived biochar (10% and 20% *v/v*) incorporated from the onset of composting on the temporal dynamics of physicochemical properties (pH, EC, water holding capacity, bulk density, particle size distribution, and organic carbon) throughout a 94-day co-composting process; (ii) characterize the dose-dependent effects of biochar on a comprehensive suite of enzyme activities linked to carbon, nitrogen, phosphorus, and sulfur cycling; and (iii) assess the suitability of the resulting compost–biochar end-products as components of soilless growing media, benchmarked against Chilean quality standard NCh 2880 [30].

2. Materials and Methods

2.1. Feedstock and Composting Process: Utilization of Biochar as an Additive

2.1.1. Feedstock

The feedstock used for composting consisted of corn (*Zea mays* L.) residues, including stover, canes, and leaves, with an initial moisture content of approximately 10%. These materials were pre-chipped (between 2 and 5 cm) to promote homogenization and facilitate microbial decomposition. Swine manure was sourced from AASA Ltd. (Nancagua, Chile; 34°38' S, 71°07' W), obtained from pigs in the breeding and weaning stages. The manure contained dietary additives not disclosed by the supplier. Initial characterization of the feedstock is presented in Table 1.

2.1.2. Biochar Production and Application

Biochar was employed as an additive in the composting matrix. It was produced via slow pyrolysis of hardwood residues (chips and sawdust) in a double-chamber furnace. The pyrolysis process was carried out at temperatures between 450 and 500 °C with a heating rate of approximately 5 °C min^{−1} and a residence time of 4 h per batch [25]. Before pyrolysis, the raw materials were oven-dried to a moisture content of 10%. Biochar was characterized, and the main properties are described in Table 2. Furthermore, before the application, biochar was sieved to obtain a particle size fraction between 0.15 and 5 mm to

ensure homogeneous incorporation into the compost mixture and to minimize potential interference with physicochemical measurements.

Table 1. Initial characterization of feedstock materials used in the co-composting process and reference values suggested by the Chilean Composting Standard (NCh 2880).

Materials	Corn	Swine	Control *	NCh 2880 **
pH	8.59	8.14	8.53	5.0–8.5
EC (mS/cm)	0.974	1.048	2.33	<3.00
Water content (%)	21.2	69.2	58.8	30–45
Total solids (%)	78.8	30.8	41.2	55–70
OM (%)	69.9	73		>20
TOC (%)	40.5	42.3		

* Equal volume mixture of corn straw and pig manure. Accepted parameters for base materials for Class A compost according to NCh 2880. ** NCh 2880 (Instituto Nacional de Normalización INN, Chile): Chilean official standard defining quality requirements and classification criteria (Class A and Class B) for compost derived from aerobic decomposition of organic matter. EC: Electrical conductivity; OM: organic matter; TOC: total organic carbon.

Table 2. Chemical characterization of forest biochar (hardwood biochar) used in the co-composting experiment.

Property	Unit	Result
pH	—	8.13
Electrical conductivity (EC)	dS/m	0.13
Organic matter (OM)	%	99.5
Water retention capacity	%	635
Total nitrogen (N)	%	0.02
Total phosphorus (P)	%	0.02
Total potassium (K)	%	0.14
Total sodium (Na)	%	0.02
Calcium (Ca)	%	0.15
Magnesium (Mg)	%	0.05
Ammoniacal nitrogen (NH ₄ ⁺ -N)	mg/kg	3.50
Nitric nitrogen (NO ₃ ⁻ -N)	mg/kg	0.70
Ammonium/nitrate ratio (NH ₄ ⁺ /NO ₃ ⁻)	—	5.00
Carbon/nitrogen ratio (C/N)	—	2376
Zinc (Zn)	mg/kg	52.0
Manganese (Mn)	mg/kg	87.9
Iron (Fe)	mg/kg	220
Copper (Cu)	mg/kg	6.71
Boron (B)	mg/kg	<LDM

LDM: below method detection limit. pH and EC determined in 1:5 extract. dS/m = mmho/cm; ppm = mg/kg.

2.1.3. Composting Process

Aerobic composting was conducted in a greenhouse facility at the Universidad de O'Higgins (San Fernando, Chile; 34°35' S, 70°59' W) over a period of 94 days. The greenhouse maintained average ambient temperatures of 18–22 °C and relative humidity of 55–75% throughout the experimental period. Composting units were placed on benches under indirect natural light conditions. Each composting unit was prepared with a mixture of materials and placed in perforated static containers (6 L) to maintain aerobic conditions. The initial mixture consisted of swine manure and corn residues in a 2:1 (v/v) ratio, adjusted to a target moisture content of 60% through manual irrigation and around a 25 C/N ratio. Temperature was recorded daily throughout the composting period. Manual turning was applied weekly during the mesophilic and thermophilic phases and biweekly during the cooling and maturation stages to ensure aeration and uniform decomposition [31].

2.2. Experimental Design

The composting experiment was conducted using a completely randomized design (CRD) with three replicates per treatment to ensure statistical reliability and reduce experimental error. Three treatments were established to evaluate the effect of biochar addition on the composting process and final compost properties: (i) Control (C), consisting of a compost mixture of corn residues and swine manure without biochar; (ii) CB1, the same compost mixture amended with 10% (*v/v*) biochar; and (iii) CB2, amended with 20% (*v/v*) biochar. The biochar was incorporated before composting to assess its influence on physicochemical and biological parameters during the entire process. Treatment details and component proportions are summarized in Table 3. The biochar application rates of 10% and 20% *v/v* were selected based on rates commonly reported in the growing media and co-composting literature, where biochar is evaluated as a structural and functional component of substrate formulations rather than as a soil amendment [18,19,31,32]. These rates represent the lower and upper bounds of a practical range that has been associated with improved substrate quality while remaining economically viable for producers.

Table 3. Description of the trials (C, CB1, and CB2) presented in *v/v* (volume/volume) of corn straw, swine manure, and biochar as additive of compost and their contribution to the final mixture (%).

Treatment	Volume (mL)	Corn Straw	Swine Manure	Biochar
Control without biochar	6000	50%	50%	-
CB1 composting mix + 10% biochar	6000	45%	45%	10%
CB2 composting mix + 20% biochar	6000	40%	40%	20%

2.3. Analysis of Physical and Chemical Properties During the Process and End-Products

2.3.1. Physical Properties

Particle size distribution was determined using a set of sieves with mesh sizes of 4.75, 3.35, 2.00, 1.18, 0.60, 0.25, and 0.15 mm. Water holding capacity (WHC) was measured following the protocols described by Nocentini et al. [18] and Tello and Vega [33]. Briefly, 6 g of each sample was saturated with distilled water on a Whatman 2 filter for 2 h. After draining, the moist weight was recorded, and the difference from the dry weight was extrapolated to 12 h. WHC was expressed as a percentage of the dry weight [18].

2.3.2. Chemical Properties

pH and electrical conductivity (EC) were determined following the method of Sadzawka et al. [34]. Briefly, 5 g of the compost sample was mixed with 25 mL of distilled water (1:5 *w/v* ratio). The pH was measured using a pH meter (Gondo PL700PV, Arquimed, Taipei, Taiwan). Subsequently, the mixture was centrifuged at 8000 rpm for 15 min. The supernatant was carefully extracted and used to measure EC with a conductivity meter.

Organic matter (OM) and total organic carbon (TOC) contents were determined via potassium dichromate and sulfuric acid digestion followed by spectrophotometric analysis at 600 nm (EPOCH 2 BSC BIOTECK, GrupoBios, Winooski, VT, USA). The procedure was conducted by adding 10 mL of 0.5 mol/L potassium dichromate to a dry compost sample, followed by 20 mL of sulfuric acid. The mixture was allowed to stand for 30 min in a fume hood, then diluted with 70 mL of distilled water and left overnight. An aliquot of the resulting solution was taken for absorbance measurement at 600 nm [34].

2.3.3. Germination Index (Phytotoxicity Test)

Phytotoxicity was assessed using the germination index (GI) according to the methods described by Zucconi et al. [35] and Luo et al. [36]. Two substrate dilutions were prepared: 1:10 *w/v* (5 g substrate: 50 mL distilled water) and 1:15 *w/v* (3 g substrate: 45 mL distilled

water), following Rozas et al. [19]. For each treatment and dilution, three Petri dishes were prepared with filter paper. Ten radish seeds (*Raphanus sativus* L.) were placed in each dish, and 10 mL of the corresponding dilution was added. Petri dishes were wrapped in aluminum foil and incubated at $20\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ for 72 h. The germination index was calculated considering the relative germination percentage and radicle growth according to the following formula:

$$RGP : \frac{\text{Number of germinated seeds in extract}}{\text{Number of germinated seeds in control}} * 100 \quad (1)$$

$$RRG : \frac{\text{Radicle length in extract}}{\text{Radicle length in control}} * 100 \quad (2)$$

$$GI : \frac{(RGP * RRG)}{100} \quad (3)$$

where RGP (1) = relative germination percentage (%); RRG (2) = relative radicle growth (%); GI (3) = germination index (%).

2.3.4. Compost Stability Analysis

The Solvita[®] test (Woods End Laboratories) was employed to assess compost stability by measuring carbon dioxide (CO₂) and ammonia (NH₃) emissions [37]. Fresh, undisturbed compost samples were placed into 50 mL plastic bottles up to the volume specified by the manufacturer. The samples were gently tapped to compact the material, after which specific Solvita[®] gel paddles for CO₂ and NH₃ detection were inserted into each bottle. The bottles were incubated at 25 °C for 4 h. After incubation, the paddles were removed and analyzed using the Solvita reader Software Version 1.6.0.10. (Bath, ME, USA), which interprets color changes to quantify the CO₂ and NH₃ released by the substrate. These measurements correlate with the stability level of the composted material. The Solvita[®] colorimetric test has been validated against direct gas measurement methods for CO₂ and NH₃ in multiple composting studies [37,38] and provides semi-quantitative indices of biological activity and compost maturity. While this method has lower precision than direct gas chromatography, it offers a rapid, standardized screening tool appropriate for comparative studies. Its accuracy is reported by the manufacturer as ± 0.5 units for the Compost Maturity Index under standard incubation conditions.

2.3.5. Elemental Composition and Metal Contents

To evaluate potential toxic elements such as heavy metals, elemental analysis was conducted via inductively coupled plasma optical emission spectrometry (ICP-OES; DV 5300, Perkin Elmer, Waltham, MA, USA). Samples underwent microwave-assisted acid digestion using modified aqua regia (nitrohydrochloric acid), following the protocol described by Medina et al. [12]. Analyses focused on the Control (C) and the highest biochar treatment (CB2) to provide contrasting insights on the effects of biochar addition during composting. The results of the elemental composition and metal content analysis are presented in Table S2 of the Supplementary Data.

2.4. Biological Activities, Enzymes Activities

To determine how biochar affects the biological activity of the composting process, enzyme activities associated with OM transformation and the cycles of nitrogen, phosphorus, and sulfur were evaluated from samples obtained at the beginning of the process and the end of composting (day 94) (Table 5). The enzyme activity of dehydrogenase (DHA) was determined using the methodology described by Camiña et al. [39], where the amount of INFT mL^{−1} (iodophenyl-nitrophenyl-phenyl tetrazolium formazan) generated

after incubation of composted material with INT substrate was quantified. The enzyme activity fluorescein diacetate hydrolysis (FDA) was determined using the methodology described by Schnurer and Rosswall [40], where the amount of fluorescein mL^{-1} produced after incubation of composted material with FDA substrate was quantified.

For acid phosphatase activity (related to P cycling), it was determined by the methodology described by Tabatabai [41], where the amount of *p*-nitrophenyl phosphate produced (*p*-NFF/g PS h^{-1}) was quantified after incubation of the composted material with *p*-nitrophenyl phosphate (*p*-NFF) substrate. The enzyme activity of urease (UA) and arginine ammonification (AA) (related to N cycling) was determined using the methodology described by Kandeler and Gerber [42] and Alef and Kleiner [43], respectively, where the amount of ammonium (N-NH_4^+) produced after incubation of the composted material with Urea and *L*-arginine as substrate, respectively. Finally, the enzyme activity of arylsulfatase (ARY) was determined by the methodology described by Alef [43] and Nannipieri et al. [44], where the amount of *p*-nitrophenyl (*p*-NF) was quantified after incubation of the composted material with *p*-nitrophenyl potassium sulfate as substrate. For all enzymes, colorimetric evaluation was performed using a microplate spectrophotometer (EPOCH2 BSC BIOTECK, GrupoBios, Winooski, VT, USA).

2.5. Statistical Analysis

To evaluate physicochemical end-product characteristics, one-way analysis of variance (ANOVA) was performed with treatment as the single factor. For enzyme activity data, a two-way ANOVA was conducted with treatment (C, CB1, CB2) and sampling time (Day 1 and Day 94) as fixed factors, along with their interaction (treatment $\times$ time). Before ANOVA, assumptions of normality of residuals and homogeneity of variances were verified using Shapiro–Wilk and Levene’s tests, respectively. When significant differences were detected ($p \leq 0.05$), Tukey’s honestly significant difference (HSD) test was applied for pairwise comparisons. Pearson correlation coefficients were calculated to assess bivariate relationships among enzyme activities and phytotoxicity indices. All statistical analyses were performed using InfoStat (version 2020) and R (version 4.2.1; R Core Team, 2022). Full ANOVA and statistical analyses tables are provided in the Supplementary Data (Tables S3 and S4); a summary table with F-values, degrees of freedom, *p*-values, and partial η^2 is also presented in the main text (Table 6). Regarding the two-way ANOVA for enzyme activities, a significant treatment $\times$ time interaction indicates that the direction or magnitude of the temporal change in enzyme activity (from Day 1 to Day 94) differed significantly among treatments, that is, biochar’s effect on enzyme dynamics was not uniform over time. A non-significant interaction indicates that the temporal trend was parallel across treatments, irrespective of biochar dose. This distinction is used explicitly when interpreting results in Section 3.6.

3. Results

3.1. Influence of Biochar on Physicochemical Properties of Compost—Temperature Profile

The composting process was closely monitored for temperature and key chemical properties, including pH and EC (Figure 1A,D). In addition, various physicochemical parameters such as PSD, BD, WHC, TOC, and the germination index (GI) of the final compost products were analyzed at the end of the 94-day experiment. These values were evaluated in the context of Chilean normative standards (NCh 2880) [30] and compared with relevant literature references to assess the suitability of the compost products as growing media components. The evolution of temperature throughout the 94-day composting period is shown in Figure S1. All three treatments exhibited temperatures consistently above ambient greenhouse conditions (18–22 °C), confirming active microbial decomposition

throughout the experimental period. An initial mesophilic phase was observed during approximately the first 14 days, during which temperatures increased gradually across all treatments. A moderate temperature peak was reached at approximately day 25, with a maximum recorded value of 47.5 °C in the Control treatment. Temperatures did not reach the typical thermophilic threshold (>55 °C) for an extended period, which is attributed to the small container volume (6 L) and the passive aeration design, consistent with the recognized limitations of bench-scale composting systems. Following the initial active phase, temperatures declined progressively, converging toward ambient values during the maturation stage (approximately days 60–94), indicating a reduction in microbial metabolic activity and increased organic matter stabilization. No consistent treatment-dependent differences in temperature profile were detected, suggesting that the two biochar rates tested did not substantially alter the overall thermal dynamics of the composting process at this scale.

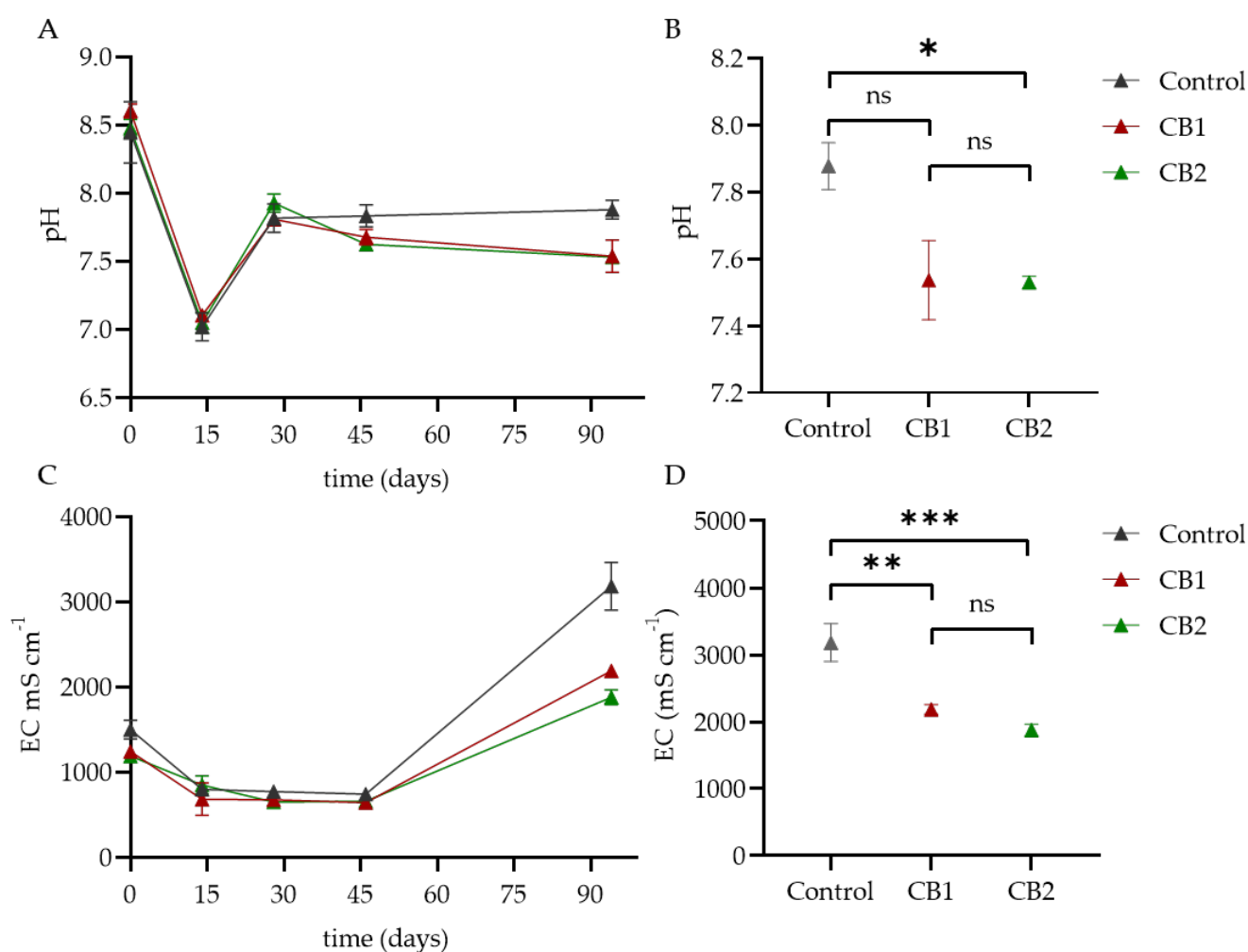


Figure 1. Change in pH and electrical conductivity by the addition of biochar to the composting process. (A) Pattern of pH change during the composting process; (B) pH compost final stage; (C) electrical conductivity (EC) of compost mixtures measured at different stages during the composting process; and (D) EC compost final stage. Mean value pH and EC (mean $\pm$ SEM, $n = 4$) on day 94, with different percentages of biochar added. CB1 corresponds to compost + 10% (v/v) biochar; and CB2 corresponds to compost + 20% (v/v) biochar. Asterisks represent statistically significant differences in a one-way ANOVA with a Tamhane T2 post hoc test (* p -value < 0.05, ** p -value < 0.01, *** p -value < 0.001). ns, indicates that there is no statistical difference. Time is expressed in days of composting.

3.2. Influence of Biochar on pH and Electrical Conductivity During Composting

Initial pH values were alkaline, exceeding 7.5 across all treatments, followed by a noticeable decline until day 14, consistent with organic acid formation during early decomposition stages. Subsequently, pH values gradually stabilized, settling between 7.5 and 7.9 by the end of composting. The Control treatment without biochar consistently exhibited the highest final pH (7.9), while biochar-amended treatments tended to maintain slightly lower but stable pH levels, which was statistically significant when 20% *v/v* of biochar (CB2) was added (Figure 1B). Electrical conductivity showed dynamic changes throughout the composting process, reflecting shifts in soluble salt concentrations. The Control treatment demonstrated the highest EC at the end of the process (3456 mS/cm). Conversely, biochar addition appeared to moderate EC increases (Figure 1C,D and Table 4).

Table 4. Physical and chemical properties of composting end-products and the reference values according to NCh 2880 [30,44]. Treatments: Control (swine manure + corn straw); CB1 (swine manure + corn straw + 10% biochar); CB2 (swine manure + corn straw + 20% biochar).

	Treatments			NCh 2880	Abad et al. [45]
	Control	CB1	CB2		
pH	7.9 a	7.5 b	7.5 b	5.0–7.5	5.2–6.3
EC (mS/cm)	3.45 a	2.18 b	1.87 b	5	0.75–3.5
BD (kg/m ³)	152.2 a	139.4 a	184.9 a	-	<400
WHC (%)	363.5 c	418.5 b	479.2 a	-	55–70
TOC (%)	31.3 a	28.3 a	27.5 a	-	-
OM (%)	53.9 a	48.9 a	47.4 a	>45	>80
Significance	***	***	***		

Notes: Values correspond to means for 3 replicates. Different letters indicate significant differences between treatments. EC: Electrical conductivity; BD: bulk density; WHC: water holding capacity; OM: organic matter; TOC: total organic carbon. Significance level: *** $p < 0.05$.

3.3. Compost Stability-Solvita[®] Respiration Test

For the analysis of compost stability, the results obtained from the Solvita[®] test, which measures compost respiration and maturity, are shown in Figure 2. CO₂ emissions (Figure 2) increased on days 28 and 94 compared to day 1. On the first day, the treatments exhibited similar emission levels. By day 28, both biochar-amended treatments showed the same percentage of CO₂ emissions, while the Control treatment, without biochar, presented the highest emission at 6%, compared to 2.89% in the biochar treatments. Regarding NH₃ emissions recorded by Solvita ©, there was a significant decrease towards the end of the composting process, reaching zero on day 94 for all treatments (Figure S2). A minor, non-statistically significant difference in NH₃ emissions was noted on the first day, with the CB2 treatment emitting 0.75 mg of NH₃ during incubation. In contrast, the Control and CB1 treatments emitted 1.03 mg and 1.08 mg of NH₃, respectively. Moreover, the compost maturity index (CMI), which is calculated by reading both probes and determining the interrelation of the two indicators (CO₂ and NH₃), showed a higher level of maturity in biochar-treated compost (≥ 6), corresponding to advanced composting being cured according to the manufacturer.

3.4. Physical and Chemical Properties of Compost End-Products

By the end of the composting period (Day 94), the incorporation of biochar significantly influenced the physical and chemical properties of the final composts ($p < 0.05$). Particle size distribution (PSD) patterns varied across treatments, with biochar-amended composts (CB1 and CB2) displaying more heterogeneous granularity, likely due to the structural contribution of biochar fragments (Table S1). Bulk density increased progressively with biochar content, from 139.4 kg/m³ in the Control to 185.9 kg/m³ in CB2, reflecting enhanced

compaction and structural integrity; however, no statistical differences were observed. Water holding capacity (WHC) showed a marked statistical increase with biochar addition, reaching 479.2% in CB2, compared to 418.5% in CB1 and 363.5% in the Control, highlighting the porous nature and sorptive properties of biochar. Meanwhile, no statistically significant differences were observed in total organic carbon (TOC) and organic matter (OM) contents, ranging from 27.5 to 31.27% and from 47.42 to 53.91%, respectively.

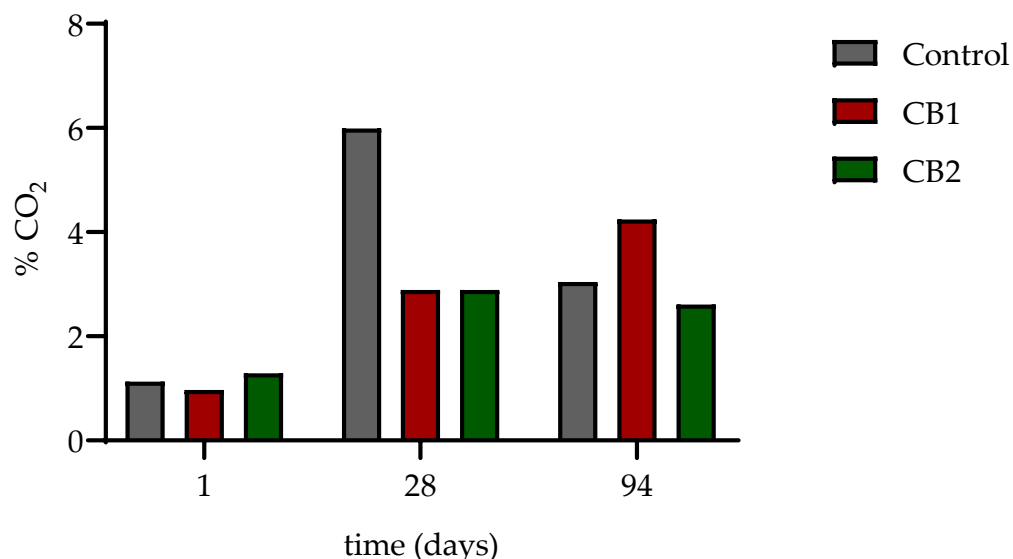


Figure 2. Solvita © test. The CO₂ (%) of compost mixtures was measured at different stages during the composting process. Time is expressed in days of composting.

3.5. Phytotoxicity Assessment—Germination Index

For the phytotoxicity analysis (Figure 3), all treatments, except for CB1 at the 1:10 dilution, exhibited higher germination rates than the Control, suggesting a general reduction in phytotoxicity following the composting process. Notably, CB2 demonstrated significantly improved germination percentages ($p < 0.01$) in both dilutions, showing significantly higher GI than CB1 and the Control within the tested range. These results indicate reduced phytotoxicity and suggest improved compost maturity in the biochar-amended treatments, particularly at 20% *v/v*. Moreover, the addition of biochar at a higher dose reduced the concentration of several metals in the compost mixtures, such as copper and zinc (146.3 and 1369.2 mg/kg, respectively), at the end of the process compared to the Control, with values of 221.5 and 2109.9 mg/kg for Cu and Zn, respectively. However, the levels of other metals such as Al and Cd were higher in comparison to the Control at the end of composting (5652 and 0.203 mg/kg, respectively (Table S2)).

3.6. Influence of Biochar on Enzyme Activities Linked to Nutrient Cycling

Two-way ANOVA revealed that biochar addition (treatment factor) had a significant effect on the activities of FDA ($F = 11.45$, $p < 0.01$), acid phosphatase ($F = 5.24$, $p < 0.05$), urease ($F = 47.56$, $p < 0.001$), arginine ammonification ($F = 27.61$, $p < 0.001$), and dehydrogenase ($F = 8.98$, $p < 0.01$), but not on arylsulfatase ($F = 1.85$, $p = 0.20$). The time factor was significant for acid phosphatase ($F = 17.95$, $p < 0.01$), urease ($F = 35.97$, $p < 0.001$), arginine ammonification ($F = 36.31$, $p < 0.001$), and arylsulfatase ($F = 5.33$, $p < 0.05$), but not for FDA or DHA. A significant treatment $\times$ time interaction was detected for acid phosphatase ($F = 7.91$, $p < 0.01$) and arginine ammonification ($F = 16.42$, $p < 0.001$), indicating that temporal dynamics differed across treatments for these enzymes (Tables 5 and 6).

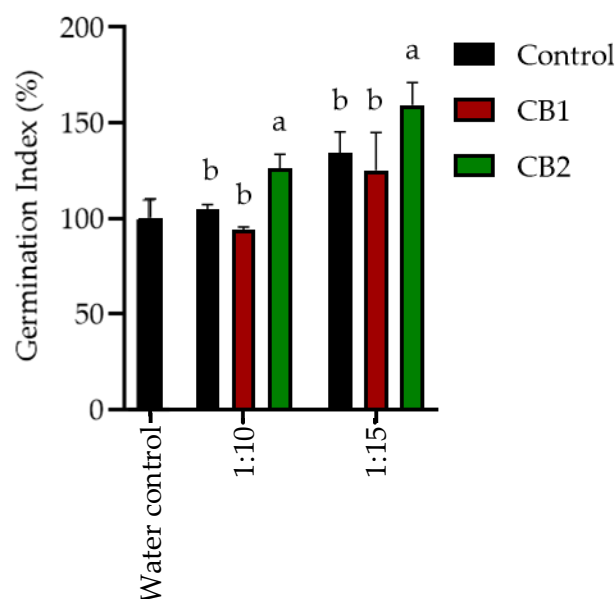


Figure 3. Germination index (GI, %) of *Raphanus sativus* seeds exposed to aqueous extracts (1:10 and 1:15 *w/v* dilutions) of compost end-products for each treatment: Control (C; swine manure + corn stover without biochar), CB1 (+10% *v/v* biochar), and CB2 (+20% *v/v* biochar). ‘Water Control’ indicates the GI obtained with distilled water used as a reference in the phytotoxicity bioassay. Different letters indicate statistically significant differences (Tukey’s HSD, $p < 0.05$). Values are means $\pm$ SEM ($n = 3$).

Table 5. Enzyme activities (mean $\pm$ SEM, $n = 3$) at Day 1 and Day 94 during co-composting of agricultural organic waste with biochar. Treatments: Control (swine manure + corn stover, without biochar); CB1 (+10% *v/v* biochar); CB2 (+20% *v/v* biochar).

Enzymes	Control		CB1		CB2		ANOVA
	D 1	D 94	D 1	D 94	D 1	D 94	
FDA $\mu\text{g Fluorescein mL}^{-1}$	1346 $\pm$ 58.8 aA	1327 $\pm$ 54.3 aA	1123 $\pm$ 134 abA	1287 $\pm$ 97.1 aA	870.0 $\pm$ 77.5 bA	994.8 $\pm$ 69.7 abA	T:F = 11.45; ** Ti:F = 1.64; ns T $\times$ Ti:F = 0.62; ns
Acid phosphatase $\mu\text{g p-NF mL}^{-1}$	16,379 $\pm$ 2600 aA	3683 $\pm$ 332 aB	11,288 $\pm$ 2099 abA	4658 $\pm$ 1903 aB	3843 $\pm$ 940 bA	5024 $\pm$ 1603 aA	T:F = 5.24; * Ti:F = 17.95; ** T $\times$ Ti:F = 7.91; **
Urease (UA) $\mu\text{g N-NH}_4^+ \text{mL}^{-1}$	1338 $\pm$ 185 aA	1918 $\pm$ 52.6 aB	757.8 $\pm$ 99.5 bA	1578 $\pm$ 92.2 aB	526.3 $\pm$ 54.3 bA	675.5 $\pm$ 91.6 bA	T:F = 47.56; *** Ti:F = 35.97; *** T $\times$ Ti:F = 5.19; *
Arylsulfatase (ARY) $\mu\text{g p-NF mL}^{-1}$	324.7 $\pm$ 153 aA	777.4 $\pm$ 367 aA	209.8 $\pm$ 35.4 aA	535.6 $\pm$ 106 aA	129.2 $\pm$ 59.6 aA	317.1 $\pm$ 25.3 aA	T:F = 1.85; ns Ti:F = 5.33; * T $\times$ Ti:F = 0.30; ns
Arg. Amm. (AA) $\mu\text{g N-NH}_4^+ \text{mL}^{-1}$	117.7 $\pm$ 27.1 aA	342.6 $\pm$ 36.1 aB	90.3 $\pm$ 5.1 aA	182.6 $\pm$ 19.7 bB	83.6 $\pm$ 6.5 aA	71.9 $\pm$ 8.5 cA	T:F = 27.61; *** Ti:F = 36.31; *** T $\times$ Ti:F = 16.42; ***
Dehydrogenase (DHA) $\mu\text{g INFT mL}^{-1}$	4279 $\pm$ 633 aA	6964 $\pm$ 1398 aB	4730 $\pm$ 527 aA	4075 $\pm$ 477 abA	2331 $\pm$ 940 aA	2218 $\pm$ 303 bA	T:F = 8.98; ** Ti:F = 0.96; ns T $\times$ Ti:F = 2.52; ns

Lowercase letters indicate significant differences between treatments at the same time point (Tukey’s HSD, $p < 0.05$). Uppercase letters indicate significant differences between Day 1 and Day 94 within the same treatment (Tukey’s HSD, $p < 0.05$). ANOVA column: T = treatment main effect; Ti = time main effect; T $\times$ Ti = interaction. ns: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Enzyme units: FDA: $\mu\text{g fluorescein mL}^{-1}$; acid phosphatase and arylsulfatase: $\mu\text{g p-NF mL}^{-1}$; urease and arginine ammonification: $\mu\text{g N-NH}_4^+ \text{mL}^{-1}$; DHA: $\mu\text{g INFT mL}^{-1}$.

Table 6. Two-way ANOVA summary for enzyme activities: F-values, *p*-values, and partial η^2 for the main effects of treatment (T), time (Ti), and their interaction (T $\times$ Ti). Values from InfoStat/R output (S5).

Enzyme	Treatment (T)			Time (Ti)			T $\times$ Ti		
	F	<i>p</i>	η^2	F	<i>p</i>	η^2	F	<i>p</i>	η^2
FDA	11.4	0.00166	0.651	1.62	0.22476	0.014	0.62	0.55237	0.094
Acid phosphatase	5.2	0.02309	0.931	17.9	0.00116	0.914	7.9	0.00643	0.916
Urease (UA)	47.5	1.98×10^{-6}	0.773	35.9	6.24×10^{-5}	0.96	5.1	0.0237	0.89
Arylsulfatase (ARY)	1.8	0.2000	0.416	5.3	0.0395	0.006	0.3	0.7460	0.923
Arginine amm.(AA)	27.6	3.24×10^{-5}	0.821	36.3	5.98×10^{-5}	0.752	16.4	0.000368	0.732
Dehydrogenase (DHA)	8.9	0.00413	0.599	0.9	0.34715	0.444	2.5	0.12240	0.807

Biochar addition emerged as the dominant factor shaping most enzymatic responses, while the influence of time and the treatment $\times$ time interaction varied across enzymes. FDA activity was significantly reduced by biochar addition ($p < 0.01$), which alone explained 70% of the variability; neither time nor the treatment $\times$ time interaction had a significant effect. FDA activity was significantly lower in CB2 compared to the Control at Day 1 (870.0 ± 77.5 vs. 1346.2 ± 58.8 $\mu\text{g Fluorescein mL}^{-1}$; $p < 0.05$), while CB1 remained intermediate (1122.5 ± 133.7 $\mu\text{g Fluorescein mL}^{-1}$) and did not differ significantly from either treatment. No significant temporal change was observed within any treatment between Day 1 and Day 94, and no treatment $\times$ time interaction was detected, indicating that the inhibitory effect of high biochar rates on general microbial activity was established from the onset and remained stable throughout composting. Acid phosphatase activity showed the most complex response pattern (treatment $\times$ time: $F = 7.91$, $p < 0.01$). At Day 1, the Control ($16,378.6 \pm 2599.8$ $\mu\text{g p-NF mL}^{-1}$) was significantly higher than CB2 (3842.5 ± 940.0 $\mu\text{g p-NF mL}^{-1}$; $p < 0.05$), with CB1 intermediate ($11,288.2 \pm 2098.8$ $\mu\text{g p-NF mL}^{-1}$). A significant temporal decline was observed in the Control and CB1 ($p < 0.05$ each), while CB2 showed no significant change over time, resulting in convergent activity values across all treatments by Day 94 (3683.1 – 5023.9 $\mu\text{g p-NF mL}^{-1}$). Urease (UA) activity increased significantly from Day 1 to Day 94 in the Control (1337.6 – 1917.9 $\mu\text{g N-NH}_4^+$ mL^{-1} ; $p < 0.05$) and CB1 (757.8 – 1578.0 $\mu\text{g N-NH}_4^+$ mL^{-1} ; $p < 0.05$), while CB2 showed no significant temporal change (526.3 – 675.5 $\mu\text{g N-NH}_4^+$ mL^{-1}). At both time points, CB2 was significantly lower than the Control ($p < 0.001$). By Day 94, CB1 recovered to activity levels not significantly different from the Control, whereas CB2 remained significantly lower than both ($p < 0.05$), consistent with the significant treatment $\times$ time interaction ($F = 5.19$, $p < 0.05$). Arginine ammonification (AA) activity increased significantly from Day 1 to Day 94 in the Control (117.4 – 342.6 $\mu\text{g N-NH}_4^+$ mL^{-1} ; $p < 0.001$) and CB1 (90.3 – 182.6 $\mu\text{g N-NH}_4^+$ mL^{-1} ; $p < 0.05$), whereas no significant temporal change was detected in CB2 (83.6 – 71.9 $\mu\text{g N-NH}_4^+$ mL^{-1}). By Day 94, significant differences were detected among all three treatments (Control $>$ CB1 $>$ CB2; $p < 0.05$), consistent with the highly significant treatment $\times$ time interaction ($F = 16.42$, $p < 0.001$). Arylsulfatase (ARY) activity was not significantly affected by biochar addition ($F = 1.85$, $p = 0.20$) or by the treatment $\times$ time interaction ($F = 0.30$, $p = 0.75$) but showed a significant increase from Day 1 to Day 94 across all treatments ($F = 5.33$, $p < 0.05$). Although mean values were numerically higher in the Control and CB1 than in CB2, no pairwise between-treatment differences reached significance at either time point, suggesting that sulfur-cycling activity responded primarily to temporal dynamics and was less sensitive to biochar dose within the range tested.

Dehydrogenase (DHA) activity was significantly reduced by biochar addition ($F = 8.98$, $p < 0.01$), with CB2 showing the lowest values at both time points. A significant temporal increase was detected only in the Control (4278.9 – 6964.4 $\mu\text{g INFT mL}^{-1}$; $p < 0.05$), while no significant temporal change was observed in CB1 or CB2. Neither the main time effect ($F = 0.96$, $p = 0.35$) nor the treatment $\times$ time interaction ($F = 2.52$, $p = 0.12$)

reached global significance, indicating that the temporal increase in DHA was limited to the Control compost.

3.7. Bivariate Relationships, Pearson's Correlation Coefficient Analysis

Figure 4 presents the Pearson correlation matrix among the analyzed variables. FDA activity, as a general indicator of overall microbial activity, showed strong correlations with acid phosphatase and urease activities ($r = 0.8$ and 0.7 , respectively) and a moderate correlation with DHA ($r = 0.6$). Acid phosphatase was significantly correlated with all other enzymatic activities except ARY and exhibited the strongest negative correlation with the germination index at 1:10 dilution (IG P 1:10; $r = -0.71$, $p < 0.05$). Arginine ammonification (AA) activity was negatively correlated with GI at 1:10 dilution ($r = -0.68$, $p < 0.05$), while DHA also showed a moderate negative association with phytotoxicity at this dilution. Urease and FDA activities were strongly intercorrelated ($r = 0.82$, $p < 0.01$), consistent with their shared microbial origin. In contrast, arylsulfatase (ARY) showed no significant correlation with phytotoxicity at either dilution tested, nor with most of the remaining enzymatic variables, suggesting a distinct response pattern relative to the other enzymes analyzed. Together, these correlations suggest two complementary pathways by which phytotoxicity was mitigated across treatments: an enzymatic pathway, where higher microbial activity (reflected in acid phosphatase and arginine ammonification) promoted organic matter mineralization and a reduction in phytotoxic compounds (Control and CB1); and a physicochemical adsorption pathway, where biochar directly adsorbed phytotoxic molecules independently of microbial activity (CB2). This mechanistic distinction is consistent with the divergent enzyme-phytotoxicity pattern observed between high- and low-biochar treatments.

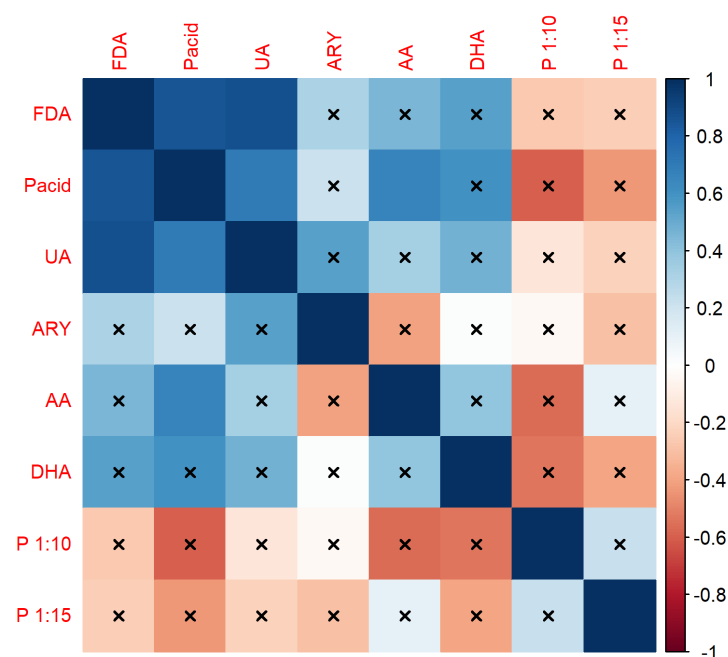


Figure 4. Bivariate correlation (Pearson's R) between the variables analyzed corresponding to phytotoxicity assay (P 1:10 and P 1:15) and enzymes evaluated. FDA: Fluorescein diacetate hydrolysis; Pacid: acid phosphatase; UA: urease; ARY: arylsulfatase; AA: arginine ammonification; DHA: dehydrogenase. Red boxes show negative correlations, whereas blue boxes show positive correlations—analysis values between -1 and $+1$. The crosses boxes indicate a statistically non-significant correlation. Non-crossed boxes show significant correlations ($p < 0.05$).

4. Discussion

4.1. Influence of Biochar Addition in Composting

The addition of biochar to composting mixtures has been extensively studied for its potential to enhance compost quality by improving nutrient retention, promoting microbial activity, and accelerating organic matter stabilization. In this study, we evaluated the chemical and enzymatic changes in compost amended with biochar derived from hardwood material.

4.1.1. Chemical Changes in Compost

Addressing objective (i) to evaluate the temporal dynamics of physicochemical properties, the results demonstrated significant dose-dependent effects of biochar on pH and EC throughout the 94-day composting process, as detailed below. According to the literature, the lower initial pH observed in this study aligns with typical patterns in regular composting operations, where acidic conditions can be attributed to multiple interconnected factors. During the early stages of biodegradation, certain bacterial groups can produce short-chain organic acids (e.g., lactic and acetic acids) as metabolic byproducts, while simultaneous microbial immobilization of NH_4^+ further contributes to pH depression [43,44,46,47]. The subsequent pH increase observed towards the end of the biodegradation process can reflect several overlapping mechanisms: the progressive degradation and consumption of organic acids, enhanced OM mineralization, and active NH_4^+ production and the subsequent conversion to NH_3 as the composting process matured [47]. At the end, the Control treatment showed significantly higher final pH (7.9) compared to the CB1 and CB2 treatments (7.5), attributed to the buffering effect of biochar [32]. Both biochar treatments were within the pH range recommended by NCh 2880, whereas the Control slightly exceeded the upper limit, indicating that biochar helped moderate alkalinity during composting [48]. The reduction in EC observed in biochar-amended treatments is consistent with previous reports of biochar's ion-adsorption capacity during composting [49,50], and is likely attributable to the binding of dissolved ionic species onto biochar surface functional groups, including carboxyl and hydroxyl moieties. In composting systems derived from swine manure, the primary contributors to EC are macronutrient-associated ions such as NH_4^+ , K^+ , Na^+ , Ca^{2+} , and Mg^{2+} arising from organic matter mineralization; however, given the characteristically high Cu and Zn loading of this feedstock, the contribution of heavy metal cations to ionic strength should not be disregarded. In this regard, the concurrent reductions in Cu and Zn concentrations observed in the CB2 end-product (Cu: 146 vs. 222 mg/kg; Zn: 1369 vs. 2110 mg/kg) suggest that co-precipitation and surface adsorption of these cations onto the biochar matrix may have contributed to the lower EC values recorded, a relationship explored further in Section 4.3. By contrast, the contribution of Al^{3+} and Cd^{2+} to EC differences is considered minor, given their relatively low concentrations and limited solubility at the measured pH range (7.5–7.9). From a growing media perspective, the EC values recorded for CB1 (2.18 mS/cm) and CB2 (1.87 mS/cm) fall within acceptable ranges for horticultural substrate formulations, while the Control (3.45 mS/cm) approached the upper threshold associated with phytotoxicity risk in sensitive species [51]. The practical implications of these findings for growing media design are discussed in Section 4.4.

4.1.2. Physical Changes in Compost

Particle size distribution analysis revealed that the CB2 treatment had the highest proportion of particles in the C range (2–0.25 mm), considered optimal for root development [45,52], while the Control showed higher proportions in the A range (>4.75 mm). This difference reflects the fine particle size of biochar material, promoting greater disintegration and improved size distribution toward smaller ranges, which can be required for substrate

formulation [53]. All treatments exhibited low bulk density ($<400 \text{ kg/m}^3$), which meets the organic substrate requirements [52]. This characteristic facilitates material handling and limits substrate compaction [54,55]. Water holding capacity (WHC) correlated inversely with particle size, with CB2 showing the highest WHC among treatments, followed by CB1 and Control [56]. Enhanced WHC in biochar treatments can result from an increased specific surface area due to the porous structure and functional groups of biochar. In this sense, the differences were statistically significant, consistent with previous findings indicating that the use of biochar obtained at higher pyrolysis temperatures tends to enhance the WHC of biochar derived from plant-based materials.

4.1.3. Compost Stability and Maturation

Solvita[®] respiration analysis showed CO_2 emissions increasing from 1% (day 1) to 6% (day 28) in the Control treatment, which can be associated with an enhanced microbial activity [38]. NH_3 emissions decreased from 1 mg (day 1) to near-zero (days 28 and 94), indicating nitrification processes under low-temperature conditions [38]. The reduction in NH_3 emissions toward process completion indicates material stabilization. The germination index (GI) exceeded 80% in all treatments and dilutions, surpassing phytotoxicity thresholds established by Emmino and Warman [57]. GI values were high in all treatments, and the biochar-amended composts, particularly CB2, consistently showed the strongest phytotoxicity reduction, supporting their suitability for horticultural applications, attributed to the capacity of biochar to adsorb NH_3 molecules among other compounds released during OM decomposition [38]. The reduced NH_3 emissions observed in Solvita[®] tests are in accordance with the high germination rates across treatments. The evaluation using 1:10 and 1:15 dilutions simulated practical substrate mixing scenarios with mineral or organic components, demonstrating the compost's suitability for horticultural applications [19].

4.2. Effect of Biochar on Enzyme Activity

Addressing objective (ii) to characterize dose-dependent effects on a comprehensive enzyme suite, the results revealed that biochar addition modulated six enzymatic activities in a complex, enzyme-specific pattern that reflects the interplay between recalcitrant carbon inputs, pH changes, and microbial community dynamics. Enzymatic activity is a sensitive indicator of microbial function and the transformation of OM during composting. Enzyme activities reflect the metabolic status of microbial populations associated with the availability of substrates [58–60]. The significant negative correlations observed between acid phosphatase and amidase activities and phytotoxicity (IG P 1:10; $r = -0.71$ and -0.68 , respectively) suggest that higher enzymatic activity, reflecting greater organic matter mineralization and nutrient cycling, is associated with a reduction in phytotoxic compounds in the compost extract. This pattern was particularly pronounced in the Control treatment at Day 94, where elevated enzyme activities coincided with lower phytotoxicity values. Notably, CB2 exhibited comparatively lower enzymatic activity yet also reduced phytotoxicity, suggesting that biochar may contribute to phytotoxicity mitigation through physicochemical adsorption mechanisms that operate independently of microbial activity. The strong intercorrelation between FDA and urease activities ($r = 0.82$, $p < 0.01$) is consistent with their shared dependence on active microbial biomass and reinforces the utility of FDA as a proxy for broader enzymatic functioning during co-composting. Notably, the divergent temporal dynamics observed between the Control and CB1 treatments showed significant increases in UA, AA, and DHA activity by Day 94, while CB2 maintained stable or declining levels, further supporting the interpretation that high biochar rates constrain the temporal development of microbial functional capacity during composting. The absence of significant correlations between arylsulfatase and phytotoxicity indicators at either

dilution suggests that sulfur-cycling enzymes are less responsive to the accumulation of organic acids and ammonia, which are primary drivers of phytotoxicity in this system, and may therefore reflect a biochemical pathway less directly coupled to compost maturity under these experimental conditions. Notably, CB2 exhibited comparatively lower enzymatic activity yet also reduced phytotoxicity, suggesting that biochar may contribute to phytotoxicity mitigation through physicochemical adsorption mechanisms that likely operate alongside, rather than instead of, microbial processes.

4.2.1. Effect of Biochar on Generalist Enzymes

The results obtained demonstrate that biochar addition presents differentiated effects on enzymatic activity during the composting process. For fluorescein diacetate (FDA) hydrolysis activity, a key indicator of active microbial biomass [29,61]. At Day 1, CB2 showed the lowest FDA activity ($870.0 \mu\text{g fluorescein mL}^{-1}$), significantly lower than the Control ($1346.2 \mu\text{g fluorescein mL}^{-1}$; $p < 0.05$), while CB1 remained intermediate. Importantly, no significant temporal change was detected within any treatment from Day 1 to Day 94, indicating that the dose-dependent inhibitory effect of biochar on general microbial activity was established from the beginning of composting and was maintained throughout the process. This pattern suggests that biochar does not so much modify the temporal trajectory of microbial activity as it sets a dose-dependent baseline that persists across the composting period. The absence of proportionality between biochar dose and FDA activity can be explained by the increase in recalcitrant carbon contribution as biochar application intensity increases. This difficult-to-decompose carbon may limit microbial activity, as reported by Chen et al. [62] and Pukalchik et al. [63]. Additionally, the intrinsic characteristics of biochar, derived from its production process (biomass type, particle size, pyrolysis temperature), generate significant changes in parameters such as pH and electrical conductivity, which are determining factors for microbial biomass activity [22]. Dehydrogenase activity (DHA) showed a treatment-dependent temporal pattern: a significant increase was observed only in the Control ($4278.9\text{--}6964.4 \mu\text{g INFT mL}^{-1}$; $p < 0.05$), while neither CB1 nor CB2 showed significant temporal changes, maintaining lower activity levels throughout the process. Overall, DHA was significantly reduced by biochar addition ($F = 8.98$, $p < 0.01$), with CB2 recording the lowest values at both time points (D1: 2331.0 ± 939.5 ; D94: $2217.6 \pm 302.9 \mu\text{g INFT mL}^{-1}$). This decrease could be linked to the reduction in fungal biomass due to the pH increase caused by biochar [64–66]. The observed decreasing trend agrees with findings by Albiach et al. [65], who attribute this decrease to the progressive depletion of nutrients and easily assimilable carbon during the composting process advancement.

4.2.2. Phosphorus and Sulfur Cycle Enzymes

Acid phosphatase activity, a crucial indicator of organic matter mineralization and phosphorus availability [65,67,68], showed a decrease toward the end of the process in all treatments except CB2. These results corroborate Chen et al.'s [68] proposal regarding the dose-dependent relationship of biochar's effect. Increased application dose may generate important pH changes that limit phosphorus availability, which tends to precipitate in different chemical forms. Variations in acid phosphatase activity are also related to biochar processing aspects, particularly pyrolysis temperature and the type of raw material used, factors that can limit phosphorus concentration in the final substrate and, consequently, enzymatic activity [69,70]. For arylsulfatase (ARY), an enzyme that catalyzes the hydrolysis of aromatic sulfated esters, an increase in activity was observed toward the end of the process in all treatments, although without significant differences between them. This behavior agrees with Li et al. [71], who reported that biochar application density favors the

water retention capacity of the substrate, increasing moisture levels, which, together with high organic matter content, favor activation and increase microbial biomass.

4.2.3. Nitrogen Cycle Enzymes

Urease (UA) activity showed a treatment-specific temporal pattern consistent with the significant treatment $\times$ time interaction ($F = 5.19$, $p < 0.05$). Significant temporal increases were observed in the Control ($1337.6\text{--}1917.9\text{ }\mu\text{g N-NH}_4^+ \text{ mL}^{-1}$; $p < 0.05$) and CB1 ($757.8\text{--}1578.0\text{ }\mu\text{g N-NH}_4^+ \text{ mL}^{-1}$; $p < 0.05$), indicating progressive nitrogen cycling capacity during organic matter decomposition in these treatments. In contrast, CB2 showed no significant temporal change ($526.3\text{--}675.5\text{ }\mu\text{g N-NH}_4^+ \text{ mL}^{-1}$; $p > 0.05$), remaining significantly lower than the Control at both time points ($p < 0.001$). This suppression of urease dynamics in CB2 is likely related to the high C/N ratio of the hardwood-derived biochar ($C/N = 2376$), which may limit nitrogen availability and thereby reduce the substrate for urease-mediated reactions [46,58,72]. Additionally, pH modification by high biochar rates may alter the ionic environment for enzyme-substrate interactions [73], while the structural pore network of biochar could compete with available nitrogen by immobilizing NH_4^+ onto surface functional groups [50].

Arginine ammonification (AA) activity exhibited the most pronounced treatment $\times$ time interaction of all enzymes evaluated ($F = 16.42$, $p < 0.001$). The Control showed the greatest temporal increase ($117.7\text{--}342.6\text{ }\mu\text{g N-NH}_4^+ \text{ mL}^{-1}$; $p < 0.001$), consistent with active ammonifying microbial populations and the progressive mineralization of organic nitrogen during composting [74,75]. The value for CB1 also increased significantly over time ($90.3\text{--}182.6\text{ }\mu\text{g N-NH}_4^+ \text{ mL}^{-1}$; $p < 0.05$), though to a lesser extent, suggesting partial preservation of ammonifying activity under moderate biochar addition. In contrast, CB2 showed no significant temporal change and remained at the lowest activity level at Day 94 ($71.9 \pm 8.5\text{ }\mu\text{g N-NH}_4^+ \text{ mL}^{-1}$), significantly lower than both the Control and CB1 ($p < 0.05$). This pattern indicates that the inhibitory effect of 20% *v/v* biochar on nitrogen-mineralizing microorganisms was sufficiently strong to prevent the temporal activation of ammonifying activity that was evident in lower-biochar treatments.

4.3. Effect of Biochar on Heavy Metal Dynamics

The following results contribute additional context to objective (iii), related to the assessment of the end-product for growing media suitability by evaluating the effect of biochar on potentially phytotoxic elements. The addition of 20% biochar (CB2) resulted in a notable reduction in Cu (146.3 vs. 221.5 mg/kg in the Control) and Zn (1369.2 vs. 2109.9 mg/kg in the Control) concentrations in the final compost. These reductions are consistent with biochar's well-documented capacity to adsorb divalent heavy metal cations through mechanisms including electrostatic attraction to negatively charged surface functional groups, complexation with organic ligands, and co-precipitation as metal hydroxides or phosphates at higher pH [28,76]. The elevated Cu and Zn concentrations in the feedstock likely originate from the dietary copper sulfate and zinc oxide supplementation commonly used in swine production [28]. Importantly, the reduction achieved in CB2 approaches the upper limit for Class A compost as defined by NCh 2880 (Cu: 300 mg/kg; Zn: 1500 mg/kg), suggesting that biochar amendment may be a practical strategy to reduce heavy metal phytotoxicity risk in composts derived from swine manure. In contrast, Al and Cd concentrations were higher in CB2 (Al: 5652 mg/kg; Cd: 0.203 mg/kg) than in the Control. The elevated Al content likely reflects the mineral composition of the hardwood feedstock and possible residual ash from the pyrolysis process, rather than contamination during composting [32]. The slight increase in Cd, while remaining below NCh 2880 limits (Cd: 3 mg/kg for Class A), warrants attention and should be characterized in the biochar

raw material in future studies. These reductions are consistent with biochar's capacity to retain divalent cations and may have contributed to the lower EC values observed in CB2. These results underscore the importance of thorough biochar characterization, including elemental impurity profiling, as a prerequisite for its use in composting systems intended for food crop production.

4.4. General Implications

The results obtained demonstrate that biochar's effect on enzymatic activity during composting does not follow a uniform pattern but depends on the enzyme evaluated, the applied dose, and the specific characteristics of the biochar used. These findings underscore the importance of considering biochar physicochemical properties and their interactions with the substrate matrix to optimize its application as an amendment in composting processes. The variability observed in enzymatic responses suggests that the selection of biochar type and dose should be made with consideration of the specific objectives of the composting process and the desired characteristics of the final product. The CB2 treatment (20% *v/v* biochar) showed consistently favorable performance across multiple quality indicators, including reduced EC, higher WHC, lower phytotoxicity, and improved compost maturity indices. These results suggest that, within the range tested (10–20% *v/v*), higher biochar rates may provide greater benefits for growing media applications. However, given that only two biochar rates were evaluated and that the study was conducted at laboratory scale under partially controlled conditions, these findings should be interpreted as indicative rather than definitive. Additional studies testing a broader range of biochar rates (e.g., 5, 10, 15, 20, 30% *v/v*) and different biochar feedstocks would be required to identify a true optimum concentration for this type of co-composting system. On the other hand, the limitations of the present study should be acknowledged. The experiment was conducted under greenhouse conditions without full climate control, meaning that ambient temperature fluctuations, although within a moderate range of 18–22 °C, may have contributed to variability in the composting rates. The small container volume (6 L) and the absence of forced aeration or temperature regulation precluded the development of a true thermophilic phase, which is a recognized limitation of small-scale composting studies [51]. Additionally, the use of swine manure containing undisclosed dietary additives (including potentially copper- and zinc-based growth promoters) introduced variability in the metal content of the feedstock, as reflected in the relatively high Cu and Zn concentrations in the Control compost. Future studies should replicate these findings at a larger scale and with multiple composting cycles to confirm the robustness of the observed biochar effects. Furthermore, the experiment was conducted as a single temporal run (one composting cycle), which limits the statistical generalizability of the findings. Replication across seasons or at different times of year would be necessary to confirm the consistency of the observed biochar effects, given the sensitivity of composting processes to ambient temperature.

Taken together and despite the study limitations, the results of this research support the hypothesis that integrating biochar into the composting of agricultural residues enhances both the composting process efficiency and the physicochemical and biological quality of the resulting product. Specifically, (i) the 10% and 20% biochar treatments significantly reduced EC and moderated pH, improving the chemical suitability of the end-product for growing media applications; (ii) water holding capacity increased significantly with biochar addition, with CB2 showing a 32% increase over the Control; (iii) phytotoxicity was reduced in biochar-amended treatments, particularly at 20% addition; and (iv) enzyme activities revealed a dose-dependent trade-off, where moderate biochar rates (10%) sustained microbial functional diversity while high rates (20%) reduced overall activity but did not compromise end-product quality. The hypothesis of this study,

that integrating biochar into the composting of agricultural residues not only enhances composting efficiency but also improves the quality of the end-product, making it a viable alternative in growing media formulations, is supported by the results with the following qualifications. (i) Regarding composting efficiency, while a clear thermophilic phase was not achieved due to the small-scale design, all treatments completed active decomposition and reached the maturation stage (according to Solvita), with biochar addition moderating pH and substantially reducing EC. (ii) Regarding end-product quality, CB1 and CB2 met the NCh 2880 standard for all evaluated parameters, and the CB2 treatment additionally showed the highest WHC and the lowest phytotoxicity index, supporting its potential as a growing media component. (iii) Regarding dose dependence, the hypothesis is confirmed within the tested range (10–20% *v/v*), but the absence of intermediate concentrations and the single-cycle design preclude identification of a true optimum. Therefore, the hypothesis is partially confirmed, with the understanding that broader dose–response studies and replication under different conditions are required to fully establish the generalizability of these findings.

5. Conclusions

This study contributes new empirical evidence on the role of biochar incorporated from the onset of co-composting in shaping the physicochemical and biological characteristics of the composting process and the resulting end-product. Three main findings are highlighted:

First, biochar addition significantly modified pH and EC dynamics in a dose-dependent manner throughout the 94-day process. The 10% and 20% *v/v* treatments reduced final EC to values within acceptable ranges for growing media formulations, and the biochar-amended treatments were closest to the pH range recommended by NCh 2880. Second, addressing objective (ii), enzyme activities showed a dose-dependent response to biochar addition, with higher biochar rates (20% *v/v*) generally reducing overall microbial activity (FDA, DHA) while sustaining or even increasing some nutrient-cycling activities (UA). This dose-dependent trade-off suggests that moderate biochar rates (10% *v/v*) better sustain microbial functional diversity during composting, whereas higher rates (20% *v/v*) may partially inhibit microbial metabolism through recalcitrant carbon inputs and pH modification. Third, addressing objective (iii), the 20% *v/v* biochar treatment showed the highest water-holding capacity (479%) and the lowest phytotoxicity index (GI > 90% in both dilutions) among the rates tested, indicating the most favorable physicochemical quality profile for growing media applications within the tested range. However, given that only two biochar concentrations were evaluated in a single composting cycle at laboratory scale, these results should be interpreted as indicative rather than definitive. Additional studies testing a broader range of biochar doses and different feedstock combinations, replicated across seasons and at a larger scale, are required to identify an optimum application rate and to confirm the consistency of the observed effects. Taken together, these findings support the hypothesis that biochar incorporated at the onset of co-composting enhances both the composting process and the physicochemical quality of the end-product, with the qualification that dose optimization is critical for balancing biological activity and substrate quality. This study provides a methodological and empirical foundation for future research on the sustainable valorization of agricultural residues and livestock by-products as components of horticultural growing media.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy16131268/s1>, Figure S1: Temperature evolution during co-composting of agricultural residues and hardwood biochar in a 94 days-composting process. Figure S2: Solvita © test. The NH₃ (%) of compost mixtures was measured at different stages during the composting process. Time is expressed in days of composting. Table S1: Particle Size Distribution

Across Different Treatments at the End of the Co-composting Process. Table S2: Multi-elemental composition of compost samples. Table S3: Physicochemical Properties Statistical Analysis. Table S4: Enzymatic activities statistical analysis.

Author Contributions: Conceptualization J.M., C.R. and R.I.C.-S.; methodology J.M., H.A., G.C. and R.M.; software R.I.C.-S. and H.A.; validation J.M., H.A., C.R. and R.I.C.-S.; formal analysis R.I.C.-S. and H.A.; investigation G.C., R.M., J.M., H.A., C.R., R.I.C.-S. and M.C.-F.; resources J.M. and H.A.; data curation H.A. and R.I.C.-S.; writing—original draft preparation G.C., R.M., J.M., H.A., M.C.-F. and U.A.-V.; writing—review and editing J.M., H.A., C.R., R.I.C.-S. and U.A.-V.; visualization, G.C., R.M., J.M., H.A. and U.A.-V.; supervision J.M. and H.A.; project administration, J.M.; funding acquisition, J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ANID through the FONDECYT Initiation projects N° 11201107 and 11241285, and Fondo Puente ID21407 (PNTE2506).

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank the company AASA Ltd. for granting the materials (swine manure) that were used in the project, for the realization of thesis projects, to the LEMiBiS laboratory for allowing the use of its facilities for the measurements that were carried out. U.A.-V. thanks the URO21991 Postdoctoral Program 2025 of the Universidad de O'Higgins.

Conflicts of Interest: The authors declare no conflict of interest.

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