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# Monitoring Soil Enzymes Activity before and after Animal Manure Application

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**Abstract:** Soil enzymes (urease, invertase, acid and alkaline phosphatase) activity in the rhizosphere of field-grown tomato plants were used to monitor the impact of soil amendments (SA) and SA mixed with biochar on soil microbial activity four months after addition of amendments. The soil treatments were sewage sludge (SS); horse manure (HM); chicken manure (CM); vermicompost (worm castings); commercial inorganic fertilizer; commercial organic fertilizer; and no-mulch (NM) native soil used for comparison purposes. Soil treatments also were mixed with 10% (w/w) biochar to investigate the impact of biochar on soil enzymes activity. The results showed a significant increase in soil urease and invertase activities after incorporation of SA to native soil. Vermicompost and HM were superior in increasing urease and invertase activity four months after their addition to native soil. Alkaline phosphatase activity fluctuated among the soil treatments, revealing some obstruction of its activity. SS amended with biochar increased acid phosphatase activity by 115% four months after SS addition. Other than alkaline phosphatase, organic manure enhanced soil biological activity (microbial biomass and release of enzymes), indicating that the use of manures, rather than inorganic fertilizers, in crop production is an affordable and sustainable agricultural production system.

**Keywords:** vermicompost; chicken manure; horse manure; sewage sludge; biochar

## 1. Introduction

Soil quality is a combination of chemical, physical, and biological characteristics that enable soils to perform a wide range of functions. It is dependent on soil biology, in which microorganisms play energetic parts in soil fertility and crop production through enzymatic activity, organic matter decomposition, and nutrient cycling. Soil amendments (SA), such as animal manures, are contributors of soil fertility. Demand for food is increasing, and much of the plant production systems will depend on fertilizers. As more sewage sludge (SS) treatment districts turn to composting as a means of sludge maintenance, and because of the rapid growth in chicken manure (CM) production, SS and CM will be available in rising quantities. Recycling wastes such as SS and CM for use as low-cost organic fertilizer could result in a positive effect on the growth and yield of a wide variety of crops and promote the restoration of ecologic and economic functions of soil.

The use of bioindicators, such as soil enzymes activity, as monitoring tools to assess soil health and potential impact of SA, have been recommended [1,2]. Soil enzymes are very sensitive to the environmental stress caused by high levels of trace metals [3,4], hormones, and antibiotics [5] in animal manures, due to their impacts on soil biological activity. Effron et al. [6] reported that soil enzyme

activity was sensitive to the pH changes, but different enzymes responded differently to the soil pH that gave positive correlations with Cu, Pb, and Zn, and negative correlations with soil enzymes.

Soil microbial biomass and enzymes activity in the rhizosphere (the soil that surrounds the plant root) are bioindicators of soil biological status. The mineralization of organic matter in soil is carried out by a large community of microorganisms and involves a wide range of metabolic processes. Positive correlation between the activity of soil enzymes and nutrient mineralization was reported in agricultural soils [7].

Urease (urea amidohydrolase, EC 3.5.1.5) hydrolyzes urea fertilizers into $NH_3$ and $CO_2$, which are associated with rise in soil pH [8], resulting in a rapid N loss to the atmosphere due to $NH_3$ volatilization [9]. Accordingly, urease activity in soil has received great attention, due to its vital role in the regulation of N supply to plants after urea fertilization. Soil urease originates mainly from plants [10] and microorganisms [11]. Invertase (β-D-fructofuranosidase) is the enzyme that splits sucrose into its two components, glucose and fructose. Invertase is available in microorganisms, animals, and plants [12]. Its hydrolysis is in both acidic and alkaline conditions [13]. The activities of urease and invertase are important in soil for releasing simple carbon and nitrogen sources for the growth and multiplication of soil microorganisms.

Phosphatases convert organic phosphate esters to orthophosphate ions [14,15] available to the plant uptake, and thus constitute an important link between biologically unavailable and bioavailable phosphorus (P) pools in the soil. Phosphatases are ubiquitous in soil and produced by microorganisms in response to low levels of inorganic phosphates. The amount of P in soil available to plants is small, about 1–5% of the total P content [16]. Acid phosphatase (EC 3.1.3.2) is found in non-mammalian species such as bacteria, fungi, parasites, and plants, and most of them share structural similarities with mammalian acid phosphatase enzymes. Accordingly, the study of soil enzymes activities indicates the potential of a soil to carry out specific biochemical reactions for maintaining soil quality.

Regarding animal waste, currently, the world generates 1.3 billion tons of municipal solid waste (biosolids) annually. By 2025, the world could generate 2.2 billion tons of biosolids per year [17]. Recycling biosolids and animal manures for use as fertilizer would reduce dependence on synthetic fertilizers and provide amendments useful for improving soil structure and nutrient status at low cost to limited-resource farmers. Incorporation of organic materials, such as municipal SS compost, into soil promotes microbiological activity [18]. There is growing evidence that soil biological parameters may have potential as early and sensitive indicators of soil ecological stress and restoration [19]. In the present investigation, soil biological activity was determined by monitoring the activity of enzymes secreted by soil microorganisms. Mierzwa-Hersztek et al. [20] measured the activity of nitrifying bacteria. Investigators [21] also measured soil microbial activity by the respiration method that quantifies $CO_2$ evolution. In another study, measurement of the soil biological activity was evaluated by determining the number of selected groups of bacteria and actinomycetes using the serial dilution method [22].

Studies also have indicated that biochar (product of a process known as pyrolysis) used as a soil amendment could increase plant nutrients, soil cation exchange capacity (CEC), soil organic matter, soil microbial activities, and nutrient availability [1,23]. Biochar application to agricultural soils has a potential for climate change mitigation and improvement of soil properties, due to its increase of CEC, nutrient and water retention, and positive influences on soil microbial communities and crop yields [24]. Biochar can abate climate change by sequestering carbon, while simultaneously providing increases in plant growth and crop yields [25,26]. In addition, Antonious et al. [27] reported that municipal SS mixed with yard waste compost produced a high marketable yield and a great number of eggplant fruits, compared to NM soil. Investigators [28] found that the use of vermicompost in agricultural production systems had increased tomato fruit elemental content, compared to NM control soil. Other investigators [29] indicated that a high yield of the Chinese cabbage was achieved by using horse manure (HM) as a soil amendment. In addition, animal manure application, such as CM and HM, to soil is proposed as a solution to the worldwide waste disposal problem.

The main objective of this investigation was to assess the impact of sewage sludge, chicken manure, horse manure, vermicompost, organic and inorganic commercial fertilizers, and biochar mixed with soil amendments on soil urease, invertase, acid phosphatase, and alkaline phosphatase activity in the rhizosphere of field-grown tomato plants. Our hypothesis is that organic fertilizers and/or biochar addition to organic fertilizers can increase soil urease, invertase, and phosphatases activities, compared to no-mulch native soil or inorganic fertilizers.

## 2. Materials and Methods

A field experiment at the University of Kentucky Horticulture Research Farm in Lexington, KY, USA was established in a randomized complete block design (RCBD). Each plot was $1.2 \times 3$ m$^2$, and the entire study area contained 42 plots (3 replicates × 14 treatments). The soil treatments were: (1) control (no-mulch NM untreated soil), (2) sewage sludge (SS), (3) horse manure (HM), (4) chicken manure (CM), (5) vermicompost (worm casting), (6) organic fertilizer (Nature Safe 10:2:8), and (7) inorganic fertilizer (Southern State 20:20:20). The soil in each of the seven treatments was also mixed with 10% (w/w) biochar obtained from Wakefield Agricultural Carbon (Columbia, MO, USA) to make a total of 14 treatments. The native soil in the experimental plots was a Bluegrass-Maury silty loam (2.2% organic matter, pH 6.2), located in the Bluegrass region (Fayette County, KY, USA). The soil has an average of 56% silt, 38% clay, and 6% sand. Properties of biochar used in this investigation were: surface area 366 m$^2$ g$^{-1}$ dry, bulk density 480.6 kg m$^{-3}$, total organic carbon 88%, N 0.27%, P 2.06 mg kg$^{-1}$, K 280 mg kg$^{-1}$, Ca 1881 mg kg$^{-1}$, Cu 2.45 mg kg$^{-1}$, Mg 558 mg kg$^{-1}$, Zn 2.09 mg kg$^{-1}$, 54% moisture, temperature 200 °C, total inorganic carbon 0.34%, particle size (<0.5 mm), pH 7.4, and Cd content of 1881 mg kg$^{-1}$. All SA were applied at 5% nitrogen (N) on dry weight basis, to eliminate variations among soil treatments due to N content [1]. Researchers [30] found that the addition of too much N to growing plants early in the season resulted in large plant size, late maturity, and plant stem damage.

SS (for example that contained 5% N) was purchased from the Metropolitan Sewer District, Louisville, KY, and applied at 2242 kg ha$^{-1}$. CM (1.1% N) was obtained from the Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky. HM (0.7% N) was obtained from the Kentucky Horse Park, Lexington, Kentucky. Vermicompost (1.5% N worm castings) was obtained from Worm Power (Montpelier, VT, USA). Organic and inorganic commercial fertilizers (10% and 20% N, respectively), were obtained from the Southern States Cooperative Stores (Lexington, KY, USA) and used at 1121 and 560.5 kg ha$^{-1}$, respectively. Soil amendments were added to native topsoil at the rate of 5% N, mixed, and rototilled to a depth of 15 cm of top soil. Seedlings of tomato, *Solanum lycopersicum* var. *marglobe*, of 70 days old were planted in raised black plastic mulch, freshly tilled soil of 42 plots, and watered using a drip irrigation system. Weeding and other agricultural operations were carried out regularly as needed. The plants were sprayed with the insecticide esfenvalerate (Asana XL) three times during the growing season at seven-day intervals, at a rate of 385.3 g ha$^{-1}$ to control the Japanese and Colorado potato beetles [30].

### 2.1. Collection and Preparation of Soil Samples

Soil samples ($n$ = 3) were collected from the rhizosphere of growing tomato plants to a depth of 15 cm. This soil depth usually contains high microbial and enzymes activity. Samples were collected using a core sampler (Clements Associates, Newton, IA, USA) equipped with a plastic liner tube of 2.5 cm inside diameter. for maintenance of sample integrity. Soil samples were air dried at room temperature, passed through a 2 mm sieve, and kept at 4 °C up to 24 h before use.

### 2.2. Soil Enzymes Analysis

For determination of soil urease activity, 5 g of soil were collected from each treatment, and 10 mL of 0.1 M phosphate buffer (pH 6.7) in 50 mL volumetric flasks were kept in an incubator at 37 °C for 24 h, and the procedure was completed as described by Tabatabi and Bremner [31]. The method was developed by measuring the concentrations of $NH_4^+$ ions released in the soil solutions by the

selective electrode method [32]. A series of standard solutions of $NH_4Cl$ covering the concentrations of 0.1–100 µg $NH_4$-N $mL^{-1}$ of water was used for calibration. Urease activity was expressed as µg $NH_4$-N released $g^{-1}$ dried soil during the incubation time [33]. Invertase activity in soil was measured by the method described by Balasubramanian et al. [34]. A standard calibration curve was obtained with each group of samples, using analytical grade glucose in the range of 10–50 µg $mL^{-1}$ glucose (Sigma Chemical Company, St. Louis, MO, USA). Acid and alkaline phosphatase activities were assessed using the method developed by Tabatabai and Bremner [35], which determines p-nitrophenol released after soil incubation with sodium p-nitrophenol phosphate solution (pH 6.7 for acid phosphatase assay, and pH 11 for alkaline phosphatase assay). Extracellular acid and alkaline phosphatase activity were assayed using a colorimetric method that involved the hydrolysis of p-nitrophenyl phosphate disodium hexahydrate (p-NPP) to p-nitrophenol (PNP), by reading the absorbance at 520 nm of the yellow color formed upon hydrolysis of p-NPP to PNP. A standard curve containing 0–50 µg $mL^{-1}$ of p-nitrophenol was used for calibration.

### 2.3. Chracteristics of Soil Amendments

Soil amendments mixed with NM native soil were collected and air dried at room temperature, sieved, and subjected to chemical analysis (Table 1). Soil samples from each plot ($n$ = 3) were mixed with double-distilled water in a soil/distilled water slurry of 1:5 (w/v) ratio. After mixing thoroughly using a magnetic stirrer, soil pH and EC were measured using a hand-held portable combination (WTW Weilheim, Germany) of glass electrode with calibrated millivolt meter (pH meter) and a conductivity meter that was standardized with a KCl solution.

**Table 1.** Selected characteristics of no-mulch (NM) native soil and soil mixed with soil amendments used for growing tomato at the University of Kentucky. South Farm (Fayette County, KY, USA).

| Soil Characteristics | Inorganic Fertilizer | CM | Organic Fertilizer | SS | Vermicompost | NM | HM |
|---|---|---|---|---|---|---|---|
| KCl Soil pH | 5.29 ± 0.11 a | 5.18 ± 0.28 a | 4.88 ± 0.14 b | 4.7633 ± 0.1 b | 4.8 ± 0.15 b | 4.71 ± 0.02 b | 4.72 ± 0.09 b |
| Soil-Water pH | 6.15 ± 0.1 a | 6.057 ± 0.26 a | 5.78 ± 0.13 b | 5.67 ± 0.1 b | 5.71 ± 0.13 b | 5.63 ± 0.02 b | 5.64 ± 0.09 b |
| P, ppm | 121.3 ± 47.9 a | 89.33 ± 6.64 a | 94.83 ± 10.32 a | 100.33 ± 10.69 a | 87.67 ± 9.46 a | 95.83 ± 10.2 a | 116. ± 50 a |
| K, ppm | 533.5 ± 96 ab | 483.8 ± 74.8 ab | 446.83 ± 10.1 bc | 327.5 ± 4.92 d | 557.3 ± 79.8 a | 336.17 ± 12.06 d | 365.5 ± 26.1 cd |
| C, ppm | 1155.3 ± 28.1 bc | 1160.8 ± 51 b | 1112.8 ± 43.9 bcd | 1050 ± 28.2 d | 1230.2 ± 27.9 a | 1091.7 ± 44.9 cd | 1067.2 ± 12.2 d |
| Mg, ppm | 135.33 ± 6.05 c | 139 ± 5.29 c | 130.67 ± 4.51 c | 131.67 ± 3.4 c | 180 ± 11.43 a | 130.33 ± 2.84 c | 150.83 ± 7.8 b |
| Zn, ppm | 5.417 ± 0.36 d | 7.117 ± 0.33 b | 6.217 ± 0.44 c | 7.867 ± 0.34 a | 6.833 ± 0.21 bc | 6.65 ± 0.82 bc | 6.617 ± 0.34 bc |
| Cd, ppm | 0.09 ± 0.01 a | 0.08 ± 0.00 ab | 0.08 ± 0.01 ab | 0.08 ± 0.01 ab | 0.08 ± 0.00 b | 0.07 ± 00 ab | 0.07 ± 0.00 ab |
| Cr, ppm | 0.04 ± 0 a | 0.04 ± 0 a | 0.04 ± 0 a | 0.04 ± 0 a | 0.04 ± 0 a | 0.04 ± 0 a | 0.04 ± 0 a |
| Ni, ppm | 0.54 ± 0.08 a | 0.57 ± 0.14 a | 0.42 ± 0.02 a | 0.54 ± 0.16 a | 0.62 ± 0.15 a | 0.46 ± 0.09 a | 0.52 ± 0.10 a |
| Pb, ppm | 6.22 ± 0.21 d | 8.217 ± 0.7 bc | 7.7 ± 0.325 c | 10.1 ± 0.66 a | 6.23 ± 0.10 d | 9.183 ± 1.17 ab | 7.53 ± 0.73 c |
| Cu, ppm | 2.96 ± 0.17 cd | 3.08 ± 0.08 cd | 3.01 ± 0.1 d | 3.42 ± 0.11 b | 3.927 ± 0.18 a | 3.22 ± 0.1 cb | 3.14 ± 0.15 cd |
| EC, µS $cm^{-1}$ | 107.37 ± 7.87 ab | 95.83 ± 14.61 b | 112.03 ± 13.23 ab | 106.4 ± 13.67 ab | 122.83 ± 8.59 a | 94.4 ± 13.1 b | 89.03 ± 14.09 b |
| N-$NO_3$, ppm | 79.33 ± 8.39 a | 18.33 ± 9.24 c | 32.67 ± 8.5 bc | 20 ± 3.46 c | 37.33 ± 11.85 b | 20.67 ± 4.51 c | 25.00 ± 6 bc |
| N-$NH_4$, ppm | 50.7 ± 56.6 ab | 66.7 ± 61.4 a | 47 ± 23.5 ab | 29.7 ± 17.9 ab | 3.667 ± 0.58 b | 5.67 ± 3.79 b | 3.33 ± 0.58 b |

Each value in the table is an average of three replicates ± standard deviation. Values accompanied by the same letter(s) in each row are not significantly different. ($P > 0.05$) using Duncan's test for mean comparison. Note that CM = chicken manure, SS = sewage sludge, NM = no-mulch control treatment, and HM = horse manure.

Soil samples were dried in an oven at 105 °C for 24 h and ground manually in a ceramic mortar and pestle to pass through a 2 mm non-metal sieve. To 1 g of each dry soil powder, 10 mL of concentrated nitric acid ($HNO_3$) were added, and the mixture allowed to stand overnight, then heated for 4 h at 125 °C on a hot plate. The mixture was then diluted to 50 mL with double-distilled water and filtered through Whatman filter paper No.1. Concentrations of metals were determined using inductively coupled plasma-mass spectrometer (ICP-MS) in standard mode following the U.S. EPA method 6020a [36] using an octopole collision cell ICP-MS (7500cx, Agilent, Santa Clara, CA, USA). All metal standards were NIST traceable. Spike metal recovery ranged from 85–100%. $NO_3$-N and $NH_4$-N were determined using the electrode method [32].

### 2.4. Statistical Analysis

Data containing soil urease, invertase, acid and alkaline phosphatases activity, and soil chemical composition were statistically analyzed using analysis of variance (ANOVA), and the means were compared using Duncan's multiple range test [37].

## 3. Results and Discussion

Our results reveal significant differences in soil urease activity between the two sampling dates before (Figure 1A), and four months after, incorporation of amendments to native soil (Figure 1B). Urease activity in soil increased 2.2 times in HM-amended soil, 3.4 times in CM-amended NM soil, 7 times in SS-amended soil, and about 88 times increase in vermicompost-amended soil, four months after the addition of amendments. In addition, biochar added to inorganic (InorgBio) and organic (OrgBio) fertilizers significantly increased soil urease activity by 28% and 22%, respectively, four months after biochar addition. Garcia et al. [16] reported an increase in soil urease activity following the addition of organic materials that promoted microbial activity. This increase in soil urease revealed the transformation of nitrogen in the soil from urea into ammonium ions ($NH_4^+$). Biochar added to NM native soil (NMBio) increased urease activity by 66%, compared to NM soil (Figure 1B), indicating the role of biochar in increasing native soil urease activity.

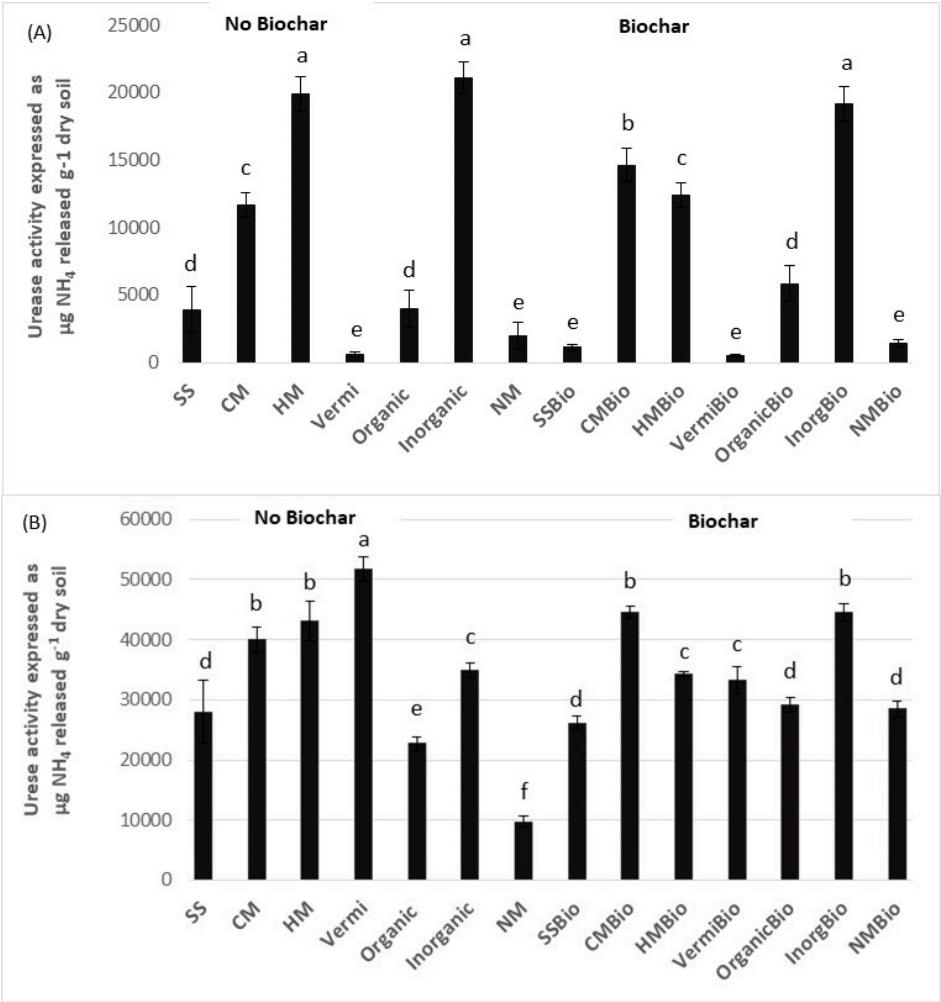


**Figure 1.** Soil amendments (SA) and biochar mixed with SA in relation to soil urease activity before (**A**), and after (**B**), treatment. Bars accompanied by different letters within each graph indicate significant differences ($P \leq 0.05$) using Duncan's multiple range test.

The electrical conductivity (EC) of vermicompost-amended soil was significantly higher by 30%, compared to the NM soil (Table 1). The increased amount of ions in vermicompost was useful in increasing the absorption of nutrients from soil, which promoted the activity of soil microorganisms and the release of urease (Figure 1B). Figure 1B also indicates that biochar added to vermicompost amended soil reduced soil urease activity.

Figure 2A indicates that soil invertase activity in CM-, HM-, CMBio-, and HMBio-amended treatments was significantly greater, compared to other amendments used in this investigation (SS, Vermi, Organic, Inorganic, NM, SSBio, VermiBio, OrganicBio, InorganicBio, and NMBio at the planting time (beginning of the field experiment). Four months after the addition of soil amendments to NM soil, significant increases in invertase activity were detected in HM-amended soil, compared to the NM control treatments (Figure 2B), reaching a maximum increase (130%) in HM-amended soil, which was superior in increasing invertase activity. In addition, biochar added to NM soil (NMBio) increased invertase activity by 52%, compared to the no-biochar native soil.

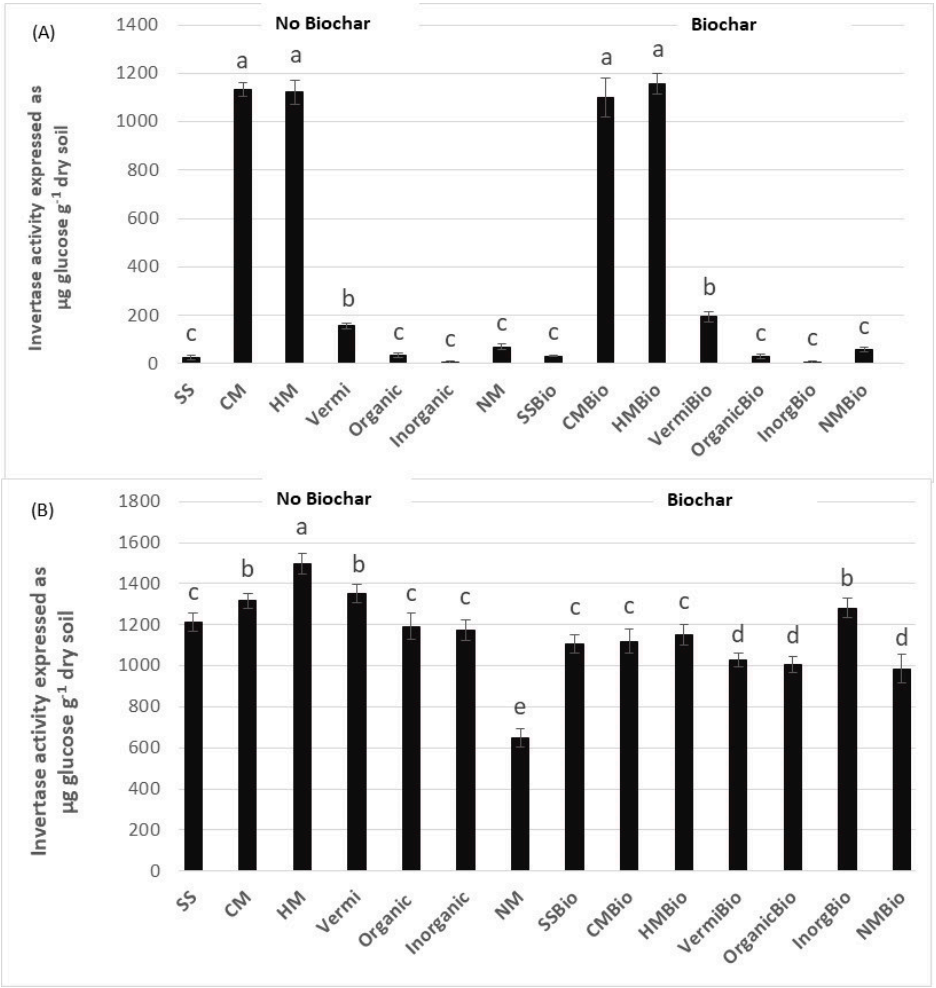


**Figure 2.** Soil amendments (SA) and biochar mixed with SA in relation to soil invertase activity before (**A**), and after (**B**), treatment. Bars accompanied by different letters within each graph indicate significant differences ($P < 0.05$) using Duncan's multiple range test.

Investigators [38] reported that the increased concentrations of Cd and Pb have negative impacts on soil microbes, and the effect of Cd on soil urease activity is more than that on invertase, while Pb has more effect on invertase activity than Cd. Table 1 indicates that there were no significant differences among the concentrations of Pb in HM-, organic fertilizer-, and CM-amended soils, indicating that the

increased activity of invertase in HM-amended soil (Figure 2B) is related to one or more other factors not investigated in our study.

Biochar is not biologically inert [20]. Aromatic carbon compounds formed during biomass pyrolysis (the process used to make biochar) are resistant to microbial decomposition, and hence the application of biochar increases the pool of carbon in the soil [39,40]. Ameloot et al. [41] and Alburquerque et al. [42] reported that biochar is an excellent source of nitrogen available not only to microorganisms, but also to plants.

SS was superior in increasing soil acid phosphatase activity. Differences among soil amendments before (Figure 3A) and after soil amendments application (Figure 3B) revealed that SS amended with native soil increased acid phosphatase activity by 8.3% and 17.7%, compared to NM no-biochar (control) and NM biochar (NMBio) treatments, respectively. SS, also known as biosolids primarily derived from domestic sources or discharges from commercial and industrial enterprises, has become less contaminated with trace metals and organic compounds. Nutrients in most synthetic commercial fertilizers are designed to be rapidly available to crops when applied to soil, which in turn increase nutrient mobility into surface runoff and infiltration water following rainfall events, whereas the organic nitrogen fraction in biosolids, such as SS, reduces the availability and mobility into runoff and infiltration water, because of its slow release of nutrients. In addition, the chemistry of inorganic nitrogen is prone to volatilization losses when surface applied; however, successful use of organic fertilizer requires adjusting application rates to account for reduced nutrient availability.

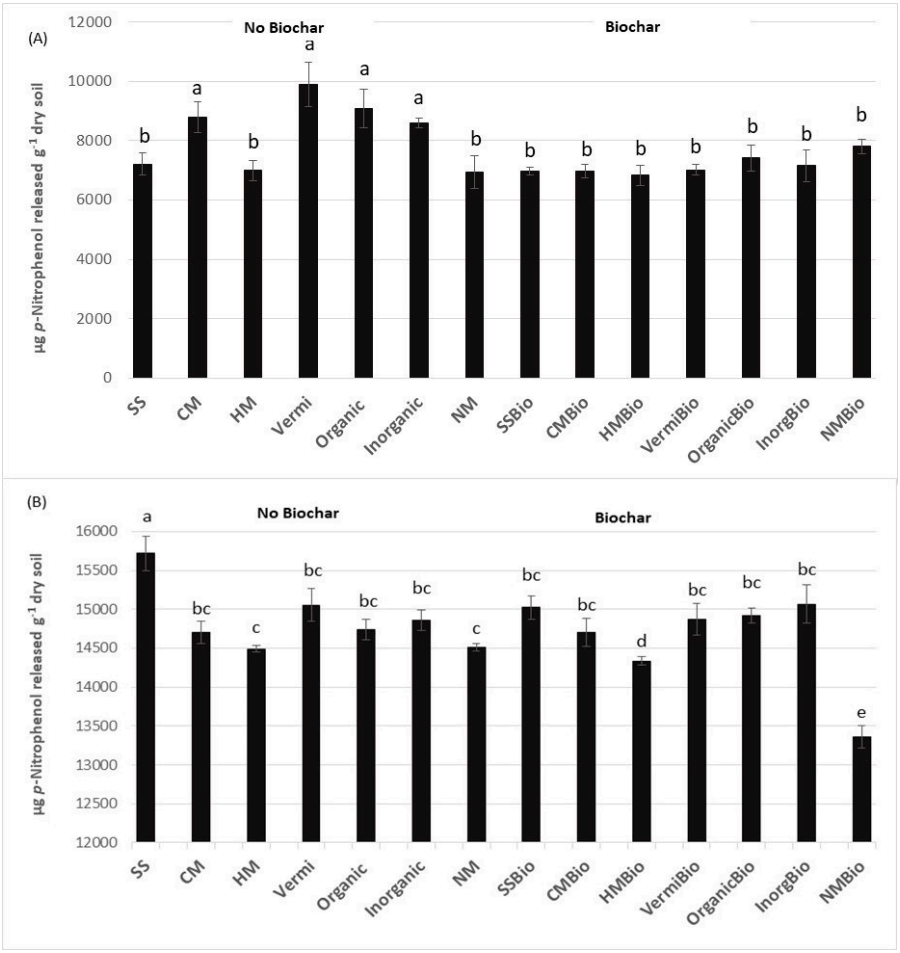


**Figure 3.** Soil amendments (SA) and biochar mixed with SA in relation to soil acid phosphatase activity before (**A**), and after (**B**), treatment. Bars accompanied by different letters within each graph indicate significant differences ($P < 0.05$) using Duncan's multiple range test.

Alkaline phosphatase activity (Figure 4A) before the incorporation of soil amendments to native soil revealed about 18% increase in CM treatment, compared to NM native soil. There were no significant differences in alkaline phosphatase activity among soil treatments in amended soils four months after treatments (Figure 4B). This could be due to the presence of other trace metals or other analytes that inhibit alkaline phosphatase activity, and/or due to the low soil pH. Data in Table 1 reveals that all soil amendments, including the NM soil, had low pH values that do not support the activity of this enzyme.

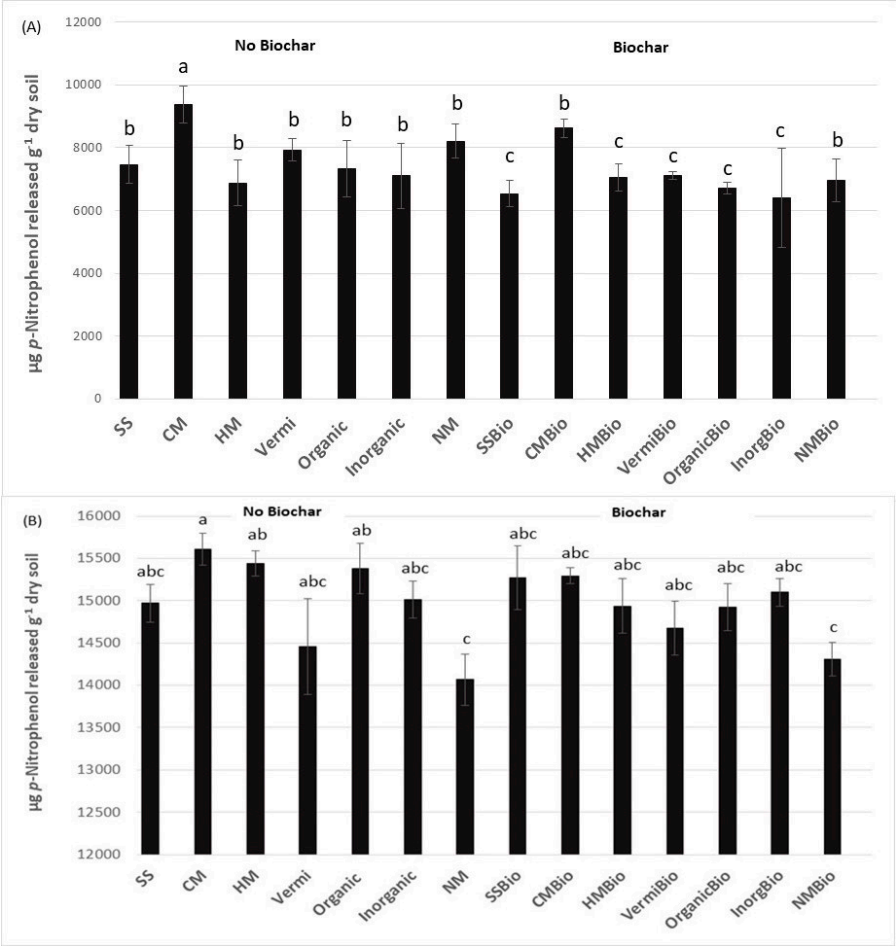


**Figure 4.** Soil amendments (SA) and biochar mixed with SA in relation to soil alkaline phosphatase activity before (**A**), and after (**B**), treatment. Bars accompanied by different letters within each graph indicate significant differences ($P < 0.05$) using Duncan's multiple range test.

Investigators reported that some analytes in animal manures, or even in native soil, act as enzyme inhibitors [43]. Berezhetsky et al. [44] indicated that the toxicity of the various metals tested toward phosphatase activity was as follows: $Cd^{2+} > Co^{2+} > Zn^{2+} > Ni^{2+} > Pb^{2+}$, due to direct interactions between trace elements and enzyme molecules, or enzymes substrates that form substrate complexes.

Table 1 reveals that no significant differences were found in Cd, Ni, and Pb between CM and NM treatments, whereas pH values were significantly greater in CM-amended soil, compared to NM soil.

In fact, many microorganisms multiplied and others were removed, due to a trace metal contamination, which resulted in shifts in the quality and functionality of soils. The potential for using recycled biosolids in agricultural production systems by adding lime (calcium carbonate) [45] or biochar [46] has been successful, due to their impacts on increasing soil pH and reducing trace metals availability to edible plants. Biosolids such as SS increased soil water retention, soil water holding capacity, and crop yield [2].

In addition, phosphatases released extracellularly by microorganisms often complex with humic compounds. Dissolved humic substances form complexes with phosphatases of bacterial and algal origin and reduce hydrolytic activity by non-competitive inhibition [47].

Tons of pharmacologically active substances are used annually in human and animal medicines for treatment and prevention of diseases. Antibiotic residues in manure, SS, and soil mixed with animal manure may affect soil microbial and enzyme activities [48]. Pharmaceutical substances used to treat farm animals do not metabolize completely within their body, they excrete in urine and feces either in their native form or as metabolites [5]. Increased fertilization of farmland with organic fertilizers such as municipal SS, CM, and HM may contribute to the introduction of antibiotics into the soil, and this might be the cause of soil phosphatase-reduced activity. There are relatively few studies on hydrolase activity and polluted soils.

Renella et al. [49] reported a reduced hydrolase activity in Cd contaminated soils. Possible causes of lower enzyme production in trace element-contaminated soils could be both microbial metabolic stress and lower mineralization of low molecular weight organic acids complexed with trace elements by soil microbial communities. More studies are needed on the effect of trace metals, hormones, and antibiotics in animal manures in contaminated soils and soil microorganisms, the enzymes they produce, and the hydrolytic activity of phosphatase in the rhizosphere of growing plants. Our data revealed that not all the amendments tested increased all enzymes activities. Biochar was not consistent in promoting all enzymes activities. We recommend the use of vermicompost to increase urease activity, HM to increase invertase activity, and SS to increase acid phosphatase activity. Soil amendments treated with biochar revealed no significant differences in alkaline phosphatase activity. Our future objectives will include a mixture of the three amendments (vermicompost, HM, and SS) to investigate their potential in elevating the activity of these three hydrolysis enzymes.

## 4. Conclusions

Soil microorganisms in the rhizosphere of growing tomato secrete a variety of extracellular enzymes. These enzymes decompose dead plants and animals, and complex forms of organic matter into accessible nutrient elements, such as C-, N-, and P-produced due to soil invertase, urease, and phosphatase activity, respectively. The effects of soil amendments (sewage sludge (SS), horse manure (HM), chicken manure (CM), vermicompost (Vermi), commercial organic (Org), and inorganic (Inorg) synthetic fertilizers) mixed with no-mulch (NM) native soil, and biochar (Bio) added to each of the soil amendments, on the activity of three soil enzymes was investigated. Significant rises in urease activity were found after the addition of some soil amendments. Data showed an increase in soil invertase activity after the addition of HM. HM was superior in increasing soil invertase activity, compared to other soil amendments tested in this investigation. Variations in amendment type and composition have a great impact on the soil microbial community and metal concentration and availability. Some analytes in animal manures or native soil act as enzyme inhibitors. Contaminated soils inhibit soil enzyme activities, especially phosphatases, which are sensitive to various inhibitors. Our results reveal that the addition of vermicompost and HM significantly ($P < 0.05$) increased the activity of urease and invertase compared to the NM treatments. The addition of biochar to SS, HM, and NM soil reduced acid phosphatase activity four months after application. Biochar added to soil amendments (CM, vermicompost, synthetic organic, and inorganic fertilizers) did not impact acid phosphatase activity. No significant differences were found in alkaline phosphatase activity among amendments treated with biochar. These results confirm the findings of other investigators, who reported that biochar has positive [20] and negative effect [50] on soil enzymes activity that might be due to the different characteristics of each of the amendments, such as variations in absorbing and retaining water molecules that impact microbial secretions. Generally, organic amendments, such as animal manures, are available at low or no cost to limited-resource farmers. For example, the total cost of nutrients required to produce 1 kg biomass of *Chlorella vulgaris* (a green microalgae used as a dietary supplement or protein-rich food additive) was estimated to be 2.5–3 USD and 60–85 USD for

using organic and inorganic fertilizer, respectively. Accordingly, utilization of available and cheap nutrient sources, such as organic fertilizers from animal manures and biosolids such as SS, rather than inorganic synthetic fertilizers, will be beneficial in large-scale crop cultivation systems in terms of cost saving and environmental quality [51].

**Author Contributions:** G.F.A. designed the study, conducted the laboratory analysis, and wrote the manuscript. E.T.T. and M.H.D. conducted the field study and project administration. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare that there is no conflict of interest.

## References

1. Antonious, G.F. Biochar and animal manure impact on soil, crop yield and quality. In *Agricultural Waste and Residue*; Intech- Open Science Books: Rijeka, Croatia, 2018.
2. Antonious, G.F. Soil amendments for agricultural production. In *Organic Fertilizers: From Basic Concepts to Applied Outcomes*; Book Chapter; Larramendy, M.L., Soloneski, S., Eds.; Intech: Rijeka, Croatia, 2016; Chapter 7; pp. 157–187. ISBN 978-953-51-4701-5.
3. Hinojosa, M.B.; Carreira, J.A.; Rodriguez-Moroto, J.M.; Garcia-Ruiz, R. Effects of pyrite sludge pollution on soil enzyme activities: Ecological dose-response model. *Sci. Total Environ.* **2008**, *396*, 89–99. [CrossRef] [PubMed]
4. Kizilkaya, R.; As¸kin, T.; Bayraki, B.; Sag˘lam, M. Microbial characteristics of soil contaminated with heavy metals. *Eur. J. Soil Biol.* **2004**, *40*, 95–102. [CrossRef]
5. Martínez-Carballo, E.; González-Barreiro, C.; Scharf, S.; Gans, O. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Ausria. *Environ. Pollut.* **2007**, *148*, 570–579. [CrossRef] [PubMed]
6. Effron, D.; de la Hora, A.M.; Defrieri, R.L.; Fontanive, V.; Palma, P.M. Effect of cadmium, copper, and lead on different enzyme activities in a native forest soil. *Commun. Soil Sci. Plant Anal.* **2004**, *35*, 1309–1321. [CrossRef]
7. Shi, W. Agricultural and Ecological Significance of Soil Enzymes: Soil Carbon Sequestration and Nutrient Cycling. In *Soil Enzymology*; Shukla, G., Varma, A., Eds.; Springer: Berlin/Heidelberg, Germany, 2011; Chapter 3; pp. 43–60.
8. Andrews, R.K.; Blakeley, R.L.; Zerner, B. Urease: A Ni (II) metalloenzyme. In *The Bioinorganic Chemistry of Nickel*; Lancaster, J.R., Ed.; VCH: New York, NY, USA, 1989; pp. 141–166.
9. Simpson, J.R.; Freney, J.R.; Wetselaar, R.; Muirhead, W.A.; Leuning, R.; Denmead, O.T. Transformations and losses of urea nitrogen after application to flooded rice. *Aust. J. Agric. Res.* **1984**, *35*, 189–200. [CrossRef]
10. Polacco, J.C. Is nickel a universal component of plant ureases? *Plant Sci. Lett.* **1977**, *10*, 249–255. [CrossRef]
11. Mobley, H.L.T.; Hausinger, R.P. Microbial urease: Significance, regulation and molecular characterization. *Microbiol. Rev.* **1989**, *53*, 85–108. [CrossRef]
12. Lef, L.K.; Nannipieri, P. *Methods in Soil Microbiology and Enzyme Activities*; Academic Press/Harcourt Brace and Company Publishers: London, UK, 1995; pp. 225–230.
13. Splading, B.P. Effect of divalent metal cations respiration and extractable enzymes activities of Douglss-fir needle litter. *J. Environ. Qual.* **1979**, *8*, 105–109. [CrossRef]
14. Alef, K.; Nannipieri, P.; Trasar-Cepeda, C. Phosphatase activity. In *Methods in Applied Soil Microbiology and Biochemistry*; Alef, K., Nannipieri, P., Eds.; Academic Press: London, UK, 1995; pp. 335–336.
15. Araujo, C.L.; Vihko, P.T. Structure of Acid Phosphatases. *Methods Mol. Biol.* **2013**, *1053*, 155–166.
16. Garcia, G.J.M.; Ocampo, J.A.; Garcıa, R.I. Enzymes in the Arbuscular Mycorrhizal Symbiosis. In *Enzymes in the Environment: Activity, Ecology, and Applications*; Burns, R.G., Dick, R.P., Eds.; Marcel Dekker, Inc.: Basel, Switzerland, 2002; Chapter 5.

17. Moya, D.; Aldás, C.; López, G.; Kaparaju, P. Municipal solid waste as a valuable renewable energy resource: A worldwide opportunity of energy recovery by using Waste-To-Energy Technologies. *Energy Procedia* **2017**, *134*, 286–295. [CrossRef]
18. Antonious, G.F. Enzyme activities and heavy metals concentration in soil amended with sewage sludge. *J. Environ. Sci. Health Part A* **2009**, *A44*, 1019–1024. [CrossRef] [PubMed]
19. Garcia, G.J.C.; Plaza, C.; Soler-Rovira, P.; Polo, A. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biol. Biochem.* **2000**, *32*, 1907–1913. [CrossRef]
20. Mierzwa-Hersztek, M.; Gondek, K.; Klimkowicz-Pawlas, A.; Baran, A. Effect of wheat and Miscanthus straw biochars on soil enzymatic activity, ecotoxicity, and plant yield. *Int. Agrophys.* **2017**, *31*, 367–375. [CrossRef]
21. Mierzwa-Hersztek1, M.; Klimkowicz-Pawlas, A.; Gondek, K. Influence of poultry litter and poultry litter biochar on soil microbial respiration and nitrifying bacteria activity. *Waste Biomass Valor* **2018**, *9*, 379–389. [CrossRef]
22. Mierzwa-Hersztek, M.; Gondek, K.; Klimkowicz-Pawlas, A.; Chmiel, M.J.; Dziedzic, F.; Taras, H. Assessment of soil quality after biochar application based on enzymatic activity and microbial composition. *Int. Agrophys.* **2019**, *33*, 331–336. [CrossRef]
23. Wu, H.; Lai, C.; Zeng, G.; Liang, J.; Chen, J.; Xu, J.; Lu, L. The interactions of composting and biochar and their implications for soil amendment and pollution remediation: A review. *Cri. Rev. Biotechnol.* **2017**, *37*, 754–764. [CrossRef]
24. Ferreira, C.; Verheijen, F.; Puga, J.; Keizer, J.; Ferreira, A. Biochar in vineyards: Impact on soil quality and crop yield four years after the application. In Proceedings of the 19th EGU General Assembly, EGU2017, Vienna, Austria, 23–28 April 2017; p. 1600.
25. Woolf, D.; Amonette, J.E.; Street-Perrott, A.A.; Lehmann, J.; Joseph, S. Sustainable biochar to mitigate global climate change. *Nat. Commun.* **2010**, *1*, 56. [CrossRef]
26. Renner, R. Rethinking biochar. *Environ. Sci. Technol.* **2007**, *41*, 5932–5933. [CrossRef]
27. Antonious, G.F.; Turley, E.T.; Sikora, F.; Snyder, J.C. Heavy metal mobility in runoff water and absorption by eggplant fruits from sludge treated soil. *J. Environ. Sci. Health* **2008**, *43*, 526–532. [CrossRef]
28. Azarmi, R.; Ziveh, P.S.; Satari, M.R. Effect of vermicompost on growth, yield and nutrition status of tomato (*Lycopersicum esculentum*). *Pak. J. Biol. Sci.* **2008**, *11*, 1797–1802. [CrossRef]
29. Laczi, E.; Apahidean, A.; Luca, E.; Dumitraş, A.; Boancă, P. *Headed Chinese* cabbage growth and yield influenced by different manure types in organic farming system. *Hort. Sci.* **2016**, *43*, 42–49. [CrossRef]
30. Pfeufer, E.; Bessin, R.; Wright, S.; Strang, J. *2018-19 Vegetable Production Guide for Commercial Growers*; University of Kentucky College of Agriculture, Food and Environment Cooperative Extension Service: Lexington, KY, USA; pp. 44–48.
31. Tabatabi, M.A.; Bremner, J.M. Assay of urease activity in soils. *Soil Biol. Biochem.* **1972**, *4*, 479–487. [CrossRef]
32. American Public Health Association (APHA). *Standard Methods for the Determination of Water and Wastewater*, 19th ed.; APHA: Washington, DC, USA, 1995.
33. Antonious, G.F. Impact of soil management and two botanical insecticides on urease and invertase activity. *J. Environ. Sci. Health* **2003**, *38*, 479–488. [CrossRef] [PubMed]
34. Balasubramanian, D.; Bagyaraj, D.J.; Rangaswami, G. Studies on the influence of foliar application of chemicals on the microflora and certain enzyme activities in the rhizosphere of *Eleusine coracana* Gaertn. *Plant Soil* **1970**, *32*, 198–206. [CrossRef]
35. Tabatabai, M.A.; Bremner, J.M. Use of p-nitrophenol phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1969**, *1*, 301–307. [CrossRef]
36. *EPA Method 6020a: Inductively Coupled Plasma -Mass Spectrometry*; USEPA: Washington, DC, USA, 1998.
37. SAS Institute Inc. *SAS/STAT Guide*; Version 6.4; SAS Inc. Campus Drive: Cary, NC, USA, 2016; p. 27513.
38. Liu, S.; Yang, Z.; Wang, X.; Gao, R.; Liu, X. Effects of Cd and Pb pollution on soil enzymatic activities and soil microbiota. *Front. Agric. China* **2007**, *1*, 85–89. [CrossRef]
39. Cheng, C.H.; Lehmann, J.; Thies, J.E.; Burton, S.D.; Engelhard, M.H. Oxidation of black carbon by biotic and abiotic processes. *Org. Geochem.* **2006**, *37*, 1477–1488. [CrossRef]
40. Lehmann, J.; Joseph, S. (Eds.) *Biochar for Environmental Management: Science, Technology and Implementation*; Routledge: London, UK, 2015.

41. Ameloot, N.; Sleutel, S.; Das, K.C.; Kanagaratnam, J.; De Neve, S. Biochar amendment to soils with contrasting organic matter level: Effects on N mineralization and biological soil properties. *Gcb Bioenergy* **2015**, *7*, 135–144. [CrossRef]
42. Alburquerque, J.A.; Calero, J.M.; Barrón, V.; Torrent, J.; del Campillo, M.C.; Gallardo, A.; Villar, R. Effects of biochars produced from different feedstocks on soil properties and sunflower growth. *J. Plant Nutr. Soil Sci.* **2014**, *177*, 16–25. [CrossRef]
43. Tyler, G.; Balsberg Pahlsson, A.M.; Bengtsson, G.; Baath, E.; Tranvik, M. Heavy-metal ecology of terrestrial plants, micro-organisms invertebrates. *Water Air Soil Pollut.* **1989**, *47*, 189–215. [CrossRef]
44. Berezhetsky, A.L.; Sosovska, O.F.; Durrieu, C.; Chovelon, J.M.; Dzyadevych, S.V.; Tran-Minh, C. Alkaline phosphatase conductometric biosensor for heavy-metal ions determination. *IRBM* **2008**, *29*, 136–140. [CrossRef]
45. Antonious, G.F.; Turley, E.; Mishra, B.; Heist, Q.; Upadhyaya, Y.; Trivette, T.; Nkuwi, L. Characterization of eggplant grown in animal manure amended soil. *Int. J. Environ. Health Res.* **2019**, *13*, 1–2. [CrossRef] [PubMed]
46. Antonious, G.; Turley, E.; Dawood, M. Ascorbic Acid, Sugars, Phenols, and Nitrates Concentrations in Tomato Grown in Animal Manure Amended Soil. *Agriculture* **2019**, *9*, 94. [CrossRef]
47. Boavida, M.J.; Wetzel, R.G. Inhibition of phosphatase activity by dissolved humic substances and hydrolytic reactivation by natural ultraviolet light. *Freshw. Biol.* **1998**, *40*, 285–293. [CrossRef]
48. Liu, F.; Ying, G.; Tao, R.; Zhao, J.L.; Yang, J.; Zhao, L. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environmental. *Environ. Pollut.* **2009**, *157*, 1636–1642. [CrossRef]
49. Renella, G.; Mench, M.; Landi, L.; Nannipieri, P. Microbial activity and hydrolase synthesis in long term Cd-contaminated soils. *Soil Biol. Biochem.* **2005**, *37*, 133–139. [CrossRef]
50. Ameloot, N.; Neve, S.D.; Jegajeevagan, K.; Yildiz, G.; Buchan, D.; Funkuin, Y.N.; Prins, W.; Bouckaert, L.; Sleutel, S. Short-term $CO_2$ and $N_2O$ emissions and microbial properties of biochar amended sandy loam soils. *Soil Biol. Biochem.* **2013**, *57*, 401–410. [CrossRef]
51. Lam, M.K.; Lee, K.T. Potential of using organic fertilizer to cultivate *Chlorella vulgaris* for biodiesel production. *Appl. Energy* **2012**, *94*, 303–308. [CrossRef]