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Variability of Biochar Performance Among Soil Amendments and Enzymes Activity

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Abstract: In a randomized complete block design, a field experiment was established with fourteen soil treatments: no mulch control (NM native soil), sewage sludge (SS), horse manure (HM), chicken manure (CM), vermicompost (Vermi), commercial organic fertilizer (Org), inorganic fertilizer (Inorg), and biochar added to NM, SS, HM, CM, Vermi, Org, and Inorg. The main objective was to assess the impact of various soil amendments (SA) and biochar added to SA on soil urease, invertase, acid and alkaline phosphatase activity involved in N, C, and P cycles, respectively. The addition of biochar to Vermi amended soil increased urease and invertase activity by 54 and 50%, respectively compared to soil mixed with Vermi alone (not amended with biochar). CM amended with biochar did not increase alkaline phosphatase activity compared to CM alone. Acid phosphatases activity decreased by about 21% after the addition of biochar to Vermi amended soil. Biochar added to HM and NM soil reduced soil alkaline phosphatase activity by 49 and 41%, respectively. The effect of soil amendments before and after the addition of biochar on soil pH, electrical conductivity (EC), nitrates (NO_3-), and ammonia (NH_4+) concentration was also investigated. No significant differences were found among soil treatments in soil pH values. Whereas Vermi mixed with biochar (VermiBio) significantly increased EC values indicating an increase in soil total ions compared to all other amendments tested. Addition of CM to native soil increased (NH_4+) ions by 7.8 times compared to the control treatment. Whereas biochar added to CM (CMBio) increased (NO_3-) ions by 2.7 times. We concluded that the duality in biochar impact on soil enzymes activity and amendments tested in this investigation requires prior testing for reconsidering the use of biochar in agricultural systems.

Keywords: Organic Materials, Microorganisms, Urease Activity, Invertase Activity, Phosphatase Activity

1. Introduction

The world generates 1.3 billion tons of municipal solid waste (MSW) annually and by 2025, the world could generate 2.2 billion tons of MSW per year [1]. The production of MSW is continuously increasing worldwide due to global urbanization of society and the increase in wastewater treatment coverage. MSW production and disposal represent a global environmental concern especially after banning of ocean disposal of biosolids. Soil amendments, such as MSW and animal manures, such as chicken manure, horse manure, and vermicompost (worm casting) are contributors of soil fertility due to their microbial content. Soil biology and fertility are dependent on soil microorganisms that impact crop production through soil enzymatic activity, organic matter decay, and nutrient availability to growing plants. Soil microbial activity is completely impacted by soil pH, soil electrical conductivity (EC), soil respiration, nitrogen (N) mineralization capacity [2] and nutrient recycling by urease, invertase, acid and alkaline

phosphatase, and other hydrolases enzymes. Application of biochar, produced by incinerating wood, as a soil amendment was proposed to enhance plant nutrients availability, soil EC, soil organic matter [3] and as potential for mitigation of climate change, retention of soil water content, and positive impact soil microbial population and consequently crop yield [4]. Studies have indicated that soil biotic properties are associated with the influence of biochar on soil C maintenance, microbial populations, and enzymatic actions [5]. In fact, the addition of biochar to soils is proposed to enhance C sequestration and microbial population in agricultural soils [6]. Accordingly, the use of soil enzymes activity as bioindicators for monitoring soil health and the potential effect of animal manures as soil amendment have been recommended [7, 8]. Soil microorganisms and their secreting enzymes are very sensitive to excessive concentrations of heavy metals [9, 10], antibiotics, hormones in animal manures [11]. Effron et al. [12] found that soil pH has a positive correlation with Cu, Pb, and Zn and negative correlation with soil enzymes activity. Monitoring soil biological factors has a potential for use as initial indicator of soil environmental stress and restoration [13]. Soil microorganisms in the rhizosphere of plants root zone secrete a variety of extracellular enzymes that decompose the remains of dead plants and animals in soil as well as complex organic matter compounds into available nutrient elements (C-, N-, P- and others) that can be absorbed by the plant roots due to soil invertase, urease, and phosphatase activity and other soil enzymes. Soil urease hydrolyzes urea as a N fertilizer and other nitrogenous compounds to produce ammonia and CO_2. Invertase hydrolyzes complex forms of C compounds to release glucose available to microorganisms, animals, and plants [14]. Invertase also gives out hydrolysis of sucrose under either acidic or alkaline conditions [15]. Phosphatase is also a hydrolytic enzyme of agricultural importance engaged in the P-cycle and is efficient in analyzing organic phosphate esters of phosphoric acid [14, 16, 17] to inorganic P and make it available to growing plants. Acid phosphatase can be found in bacteria, fungi, parasites, and plants.

Biochar application to agricultural soil has been recommended for abating climate change due to its impact on carbon (C) sequestration while promoting crop yield [18]. The biochar production process is unique because it takes more C out of the atmosphere than it releases. Small amount of C is released back into the air during the pyrolysis process and the rest is sequestered or locked up for long periods in the form of biochar. Biochar increases soil water- and nutrient-holding capacities, which typically result in increased plant growth [19]. In addition, biochar helps to provide suitable habitat for soil microbes to allow them to decompose soil organic matter [20]. Li et al. [21] also reported that biochar addition to plants seedling bed enhanced the efficiency of Cd remediation in soil after the transplantation of eggplant, *Solanum melongena* L. in sewage sludge (SS) biochar amended soil.

Investigators recognized that the application of a mixture of yard waste and SS compost enhanced agricultural soil quality [22] and supplied the most marketable eggplant yield. The addition of vermicompost to native agricultural soil improved tomato yield and nutrient content of tomato fruits [23]. Soil amended with horse manure provided the most Chinese cabbage yield [24]. Antonious [8] also reported that application of municipal SS, chicken manure, and horse manure to native soil is a potential solution to the waste disposal problem and provides amendments at affordable costs to limited resource farmers due to the value of this waste as alternative to inorganic fertilizers that have several side effects on natural water resources, such as eutrophication (high concentrations of N and P). Accordingly, measuring soil pH, electrical conductivity (EC), nitrates (NO_3), and ammonia (NH_4) ions, and soil enzymes activity provide soil information on the nutrient availability to growing plants. These parameters can also be indicators of the effect on soil biological activity.

The main objective of this investigation was to assess the impact of soil amendments: sewage sludge (SS), horse manure (HM), chicken manure (CM), vermicompost (Vermi), commercial organic fertilizer (Org), inorganic fertilizer (Inorg), and biochar added to native soil, SS, HM, CM, Vermi, Org, and Inorg on soil urease, invertase, acid and alkaline phosphatase activity involved in N, C, and P cycles, respectively.

2. Materials and Methods

2.1. Field Work

The field experiment was designed and applied at the University of Kentucky Horticulture Research Farm (Fayette County, KY, USA in a randomized complete block design (RCBD). The native soil in the experimental plots is a Bluegrass-Maury Silty Loam (2.2% organic matter, pH 6.2) located at the blue grass region (Fayette County, KY). The soil in the experimental area consists of 56% silt, 38% clay, and 6% sand. Each plot was 4 × 10 ft^2 (1.23 × 3.05 cm^2) and the entire study area contained 42 plots (3 replicates × 14 treatments). The soil treatments used in this investigation were: control (no-mulch NM native soil), sewage sludge (SS), horse manure (HM), chicken manure (CM), vermicompost (Vermi), organic (Org) fertilizer (Nature Safe 10N: 2P: 8K), inorganic (Inorg) fertilizer (Southern State 19N: 19P: 19K), and biochar added to NM soil, SS, HM, CM, Vermi, Organic, and Inorg fertilizers). Biochar obtained from Wakefield Agricultural Carbon (Columbia, MO) was mixed with soil amendments at 10% (w/w) in seven treatments, replicated three times. 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 C 88%, moisture 54%, temperature 200°C, total inorganic C 0.34%, particle size (<0.5 mm), pH 7.4, and 1881 mg kg^{-1} calcium content. All soil amendments were applied at 5% nitrogen (N) on dry weight basis to eliminate variations among soil treatments due to N content [7]. SS was purchased from the Metropolitan Sewer District, Louisville, Kentucky, and CM was obtained from the

Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky. HM was obtained from the Kentucky horse park, Lexington, Kentucky. Vermi (worm castings) was obtained from Worm Power (Montpelier, Vermont, USA) and Org and Inorg commercial fertilizers were obtained from the Southern States Cooperative Stores (Lexington, Kentucky). Soil amendments were mixed with the top native topsoil and rototilled to a depth of 15 cm of topsoil. Eggplant, *Solanum melongena v*ar. Epic seedlings of sixty days old were planted in a freshly tilled soil at 18 inches (45 cm) in-row space and drip irrigated as needed. Weeding and other agricultural practices were applied as needed. The plants were sprayed with the insecticide esfenvalerate (Asana XL) three times during the growing season at a rate of 385.3 g ha^{-1} to control Japanese and Colorado potato beetles [25].

2.2. Collection and Preparation of Soil Samples

Soil samples (n=3) were collected after planting from each treatment from the rhizosphere of growing plants to a depth of 15 cm (a zone of increased microbial and enzyme activity where soil and root make contact). Samples were collected using a core sampler (Clements Associates, Newton, IA) equipped with a plastic liner tubes of 2.5 cm i.d. for maintenance of sample integrity. Soil samples were air-dried at room temperature, passed through a 2 mm non-metallic sieve, and kept at 4°C C up to 24 h before use.

2.3. Soil Enzymes Analysis

Soil urease activity was determined by collecting a five-g of soil from each treatment and adding 10 mL of 0.1 M phosphate buffer (pH 6.7) in 50 mL volumetric flasks. The flasks were incubated at 37°C for 24h and the procedure was completed as described by Tabatabi and Bremner [26]. Concentrations of NH_4^+ ions released after incubation in the soil solutions were determined by the selective electrode method [27]. Standard solution of NH_4Cl at the concentrations of 0.1-100 µg NH_4-N mL^{-1} of water was used for standardization. Urease activity was expressed as µg NH_4-N released g^{-1} dried soil [28]. Invertase activity in soil was estimated by the method described by Balasubramanian et al. [29]. A standard calibration curve was used 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 activity in soil were determined by the method developed by Tabatabai and Bremner [30] which determines p-nitrophenol released when soil is incubated with sodium p-nitrophenol phosphate solution at pH 6.7 for acid phosphatase measurement and pH 11 for alkaline phosphatase measurement. The method involved the hydrolysis of *p*-nitrophenyl phosphate disodium hexahydrate (*p*-NPP) to p-nitrophenol (PNP) by phosphatase and reading the absorbance 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.

2.4. Soil pH and Conductivity

Representative soil samples were collected from each plot (n=3) and mixed with double distilled water in a soil: distilled water slurry of 1:5 (w/v) ratio. A magnetic stirrer was used for mixing the soil solutions and the pH and EC values were determined using a hand-held portable combination of glass electrode with calibrated pH millivolt meter and a conductivity meter (WTW Weilheim, Germany).

2.5. Potentially Mineralizable Nitrogen Analysis

Soil mineralizable N content was analyzed as described by Gianello and Bremmer [31] in which 20 mL of 2.0 N KCl was added to 3 g of air-dried soil in glass centrifuge tubes, placed in a block digester (Benchmark, Digital dry Bath II, BSH1004) and incubated at 100°C for 4 hrs. Soil mineral N in the form of NO_3^- and NH_4^+ ions in the samples filtrate was determined using a microplate spectrometer (Epoch Model, BioTek Instruments, Inc., Winooski, VT) [32]. A series of standard solutions of ammonium nitrates (NH_4NO_3) covering the concentrations of 0 – 10 µg mL^{-1} of water was used for calibration.

2.6. Statistical Analysis

Data containing soil urease, invertase, acid and alkaline phosphatase activity, soil pH, conductivity, nitrates (NO_3-), and ammonia (NH_4+) ions were statistically analyzed using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test [33].

3. Results and Discussion

Urease activity in the rhizosphere of eggplant varied due to the different types of soil amendments mixed with native soil. The activity of soil urease indicates soil N cycling that was significantly higher ($P \leq 0.05$) in Vermi mixed with biochar (VermiBio) treatments compared to Vermi treatments with no biochar. Similarly, biochar added to HM (HMBio) was effective in promoting urease activity (Figure 1) indicating the role of biochar in promoting soil urease activity. On the contrary, biochar added to SS (SSBio), CM (CMBio), organic (OrgBio), inorganic (InorgBio) commercial fertilizers, and no-mulch native soil (NMBio) did not increase soil urease activity compared to the no biochar treatments. Studies have shown that the activities of soil enzymes might be inhibited by trace metals [34] in biochar. The loss of organic matter during the biochar preparation process (pyrolysis) contributes to an increase in the concentration of trace metals in biochar. Zn for example inhibited urease activity [35]. However, Ameloot et al. [36] and Alburquerque et al. [37] reported that biochar is an excellent source of N available not only to microorganisms, but also to plants.

Several researchers recognized the dual efficiency of biochar added to agricultural soils. In some experiments, biochar decreased crop productivity [38-40], whereas other studies revealed that biochar showed no impact on soil inorganic N retention or loss [41-43]. Others reported that the application of biochar to soil speeded soil nitrification

(transformation of ammonia to nitrite) [44, 45]. These conflicting results might be attributed to the fact that the sorption of N fractions on biochar particles varied with the type of biochar prepared from different resources as well as to the differences in its production conditions [46, 47]. These variations make biochar impact on soil microbial communities either, harmful, helpful or insignificant depending on the source of biochar as well as the type of soil used in monitoring its impact [48-50]. Variability also could be mainly due to the type of soil. Soil particles pores differ among soil types. A sandy soil for example has specific surface area of 0.01–0.1 m^{-2} g whereas a clay soil has specific surface area of 5–700 m^{-2} g soil [51]. In addition, biochar showed higher specific area, ranging from 0.5 to 2200 m^2 g^{-1} as a function of heating temperature used during its preparation [52]. Accordingly, soil porosity is a critical factor in controlling the rate at which water infiltrates through the soil particles. Small soil pores retain soil solution and nutrients by capillarity action, and this reduces nutrient leaching through the soil column [53] due to nutrient entrapment.

Invertase, the enzyme involved in the C cycle, also plays an important role in increasing soluble nutrients in soil and is often used to monitor and characterize soil fertility. Figure 2 revealed that VermiBio was superior in increasing soil invertase activity from 3970 to 5,947 µg g^{-1} dry soil (about 50% increase) compared to Vermi with no biochar addition, indicating the role of biochar in promoting invertase activity in VermiBio treatments. Whereas no significant differences were found among other treatments in promoting invertase activity. However, there is evidence that organic materials promote microbial activity [13] and their enzyme secretions. Ren et al. [54] reported that the major source of soil organic carbon (SOC) in agricultural soils is organic manure and an increase in SOC content is generally expected following land application of animal manure. Christopher and Lal [55] indicated that that the application of mineral N fertilizer increased crop production, plant root exudates, and enhanced SOC sequestration in agricultural soils. Liu et al. [56] also reported that the application of organic manure improved SOC. In addition to increased soil C concentrations in soils handled organically compared to soils managed by conventional farming was reported [57-59], whereas such variation had not been found by some other agricultural researchers [60, 61].

Soil acid phosphatase activity was significantly reduced by 43% due to addition of commercial Inorg fertilizer (Southern State NPK 19: 19: 19) to no-mulch (NM) native soil, whereas soil amended with commercial Org fertilizer (Nature Safe NPK 10: 2: 8) increased acid phosphatase activity by only 7% compared to NM soil (Figure 3A). The activity of soil acid phosphatases dropped by 21% (from 1414 to 1124 µg g^{-1} dry soil) following the addition of biochar to Vermi amended soil (VermiBio). On the other hand biochar added to SS amended soil (SSBio), Org amended soil (OrgBio), and NM soil (NMBio) soil did not alter acid phosphatase activity compared to SS, Org, and NM treatments not amended with biochar (Figure 3A).

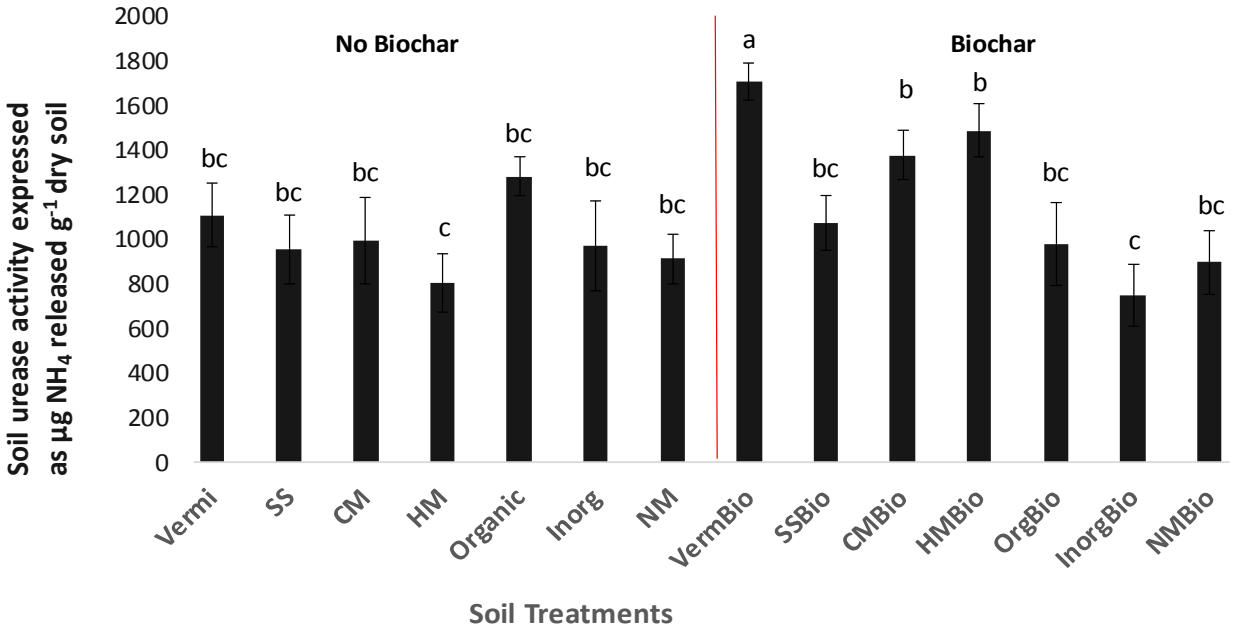

Figure 1. *Impact of soil amendments (SA) and SA mixed with biochar on soil urease activity in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars ± standard deviation accompanied by different letter (s) indicate significant differences (P≤0.05) using Duncan's multiple range test.*

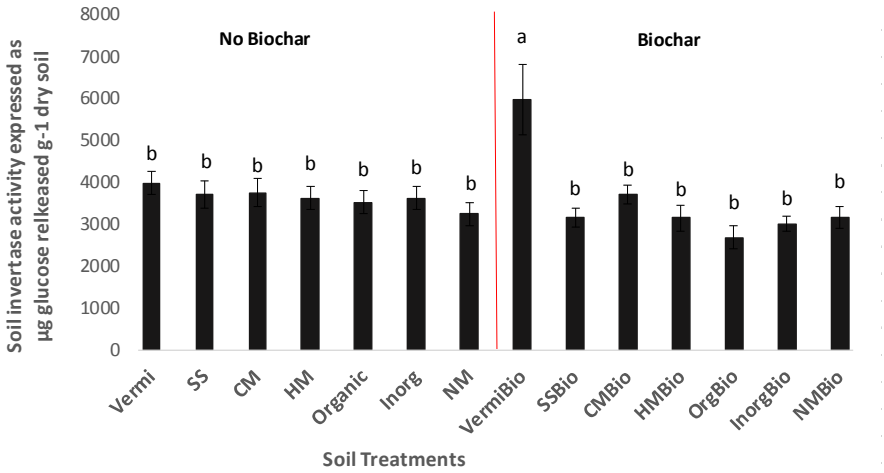

Figure 2. *Impact of soil amendments (SA) and SA mixed with biochar on soil invertase activity in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars ± standard deviation accompanied by different letter indicate significant differences (P≤0.05) using Duncan's multiple range test.*

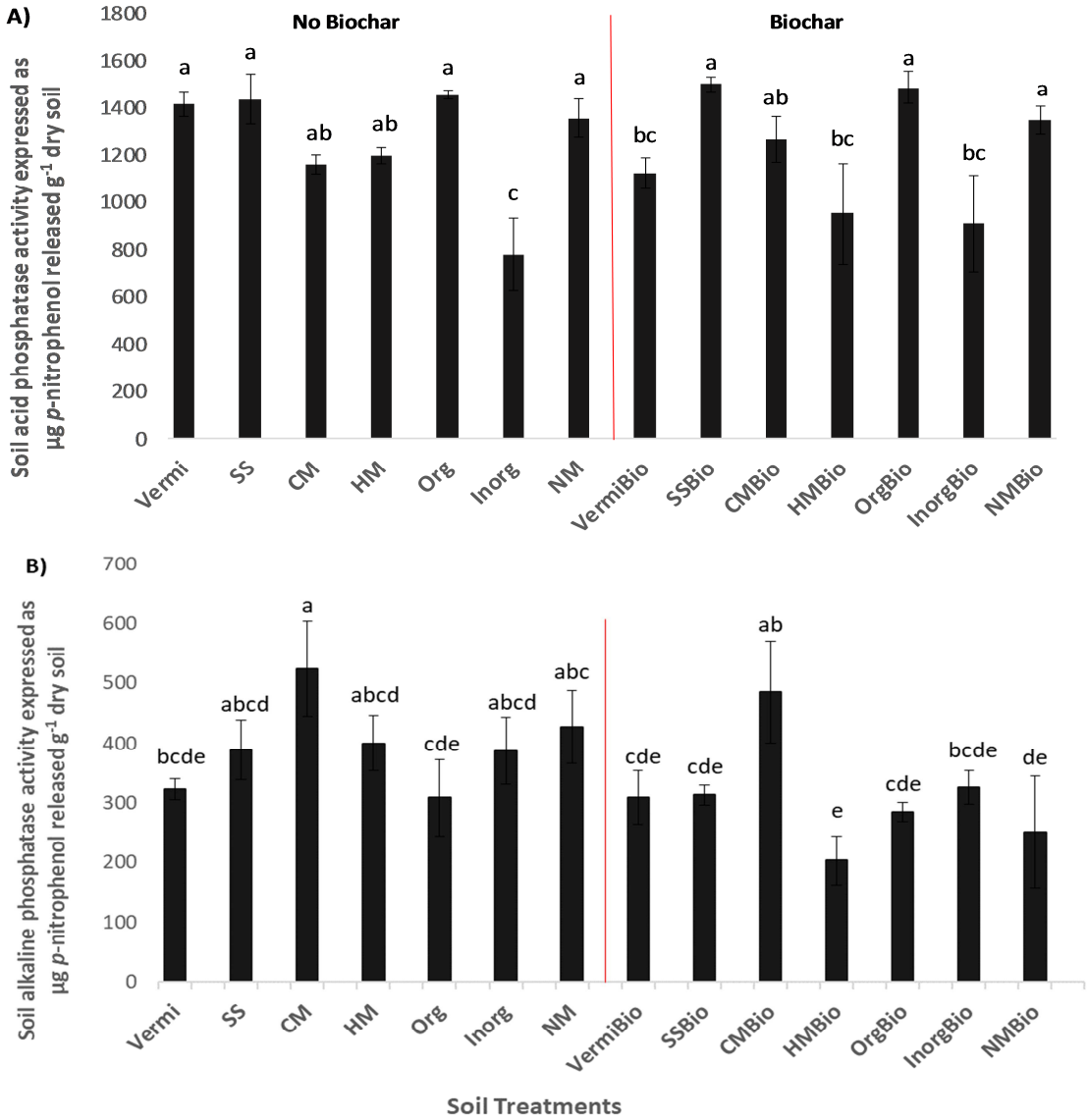

Figure 3. *Impact of soil amendments (SA) and SA mixed with biochar on soil acid phosphatase activity (A) and alkaline phosphatase activity (B) in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars ± standard deviation accompanied by different letter (s) indicate significant differences (P≤0.05) using Duncan's multiple range test.*

No significant differences were found in soil alkaline phosphatase activity between Org and Inorg fertilizers amended or not amended with biochar (Figure 3B). Results also revealed that biochar added to CM (CMBio) did not increase alkaline phosphatase activity compared to CM treatments not amended with biochar. Biochar added to no mulch soil (NMBio) reduced soil alkaline phosphatase activity by 41% compared to NM native soil not amended with biochar. One possible reason is that biochar added to soil amendments might contain one or more alkaline phosphatase inhibitors. Many soil microorganisms multiply and others removed, due to a trace metal contamination, which results in shifts in the quality and functionality of soils. Metals in animal manures and some types of soils act as enzyme inhibitors [62]. Phosphatases also were impacted by metal cations, such as Mg^{2+} and Ca^{2+} required for their activity [63]. Berezhetsky et al. [64] indicated that the toxicity of the various metals tested toward immobilized phosphatase was in the order: $Cd^{2+} > Co^{2+} > Zn^{2+} > Ni^{2+} > Pb^{2+}$ due to direct interactions between metals and enzyme molecules, or enzymes substrates that form substrate complexes. Cd significantly inhibited alkaline phosphatase activity, whereas Zn inhibited urease activity [35].

Soil pH and electrical conductivity (EC) provide information on the nutrient availability to growing plants and can also be used as indicators of the effects of soil properties on soil biological activity and microbial mediated processes. Most plant nutrients are readily available within the pH range of 6.0 to 7.5 and higher crop yields were achieved in the pH range of 5.7 to 7.5 since the optimum pH range for most soil microorganisms is the range of 5 to 8 [65]. Figure 4A revealed that soil pH was not significantly different among soil treatments amended with biochar or treatments that have not amended with biochar. Alburquerque et al. [37] found that the application of biochar in doses below 1% in slightly acidic soil, did not affect soil pH. Figure 4B revealed significant variations in soil EC values among treatments. Biochar added to Vermi (VermiBio) increased soil EC by 9.7%, whereas EC values fluctuated among the other treatments, but were not significantly impacted by biochar addition. Soil EC values are controlled by the presence of cations and anions in the soil solution, which in turn impact soil salinity. Soil salinities influence physical, chemical, and biological properties in soil due to the variability of. microorganisms in their tolerance to salt (actinomycetes and fungi are more tolerant to higher soil EC values, whereas bacteria are sensitive).

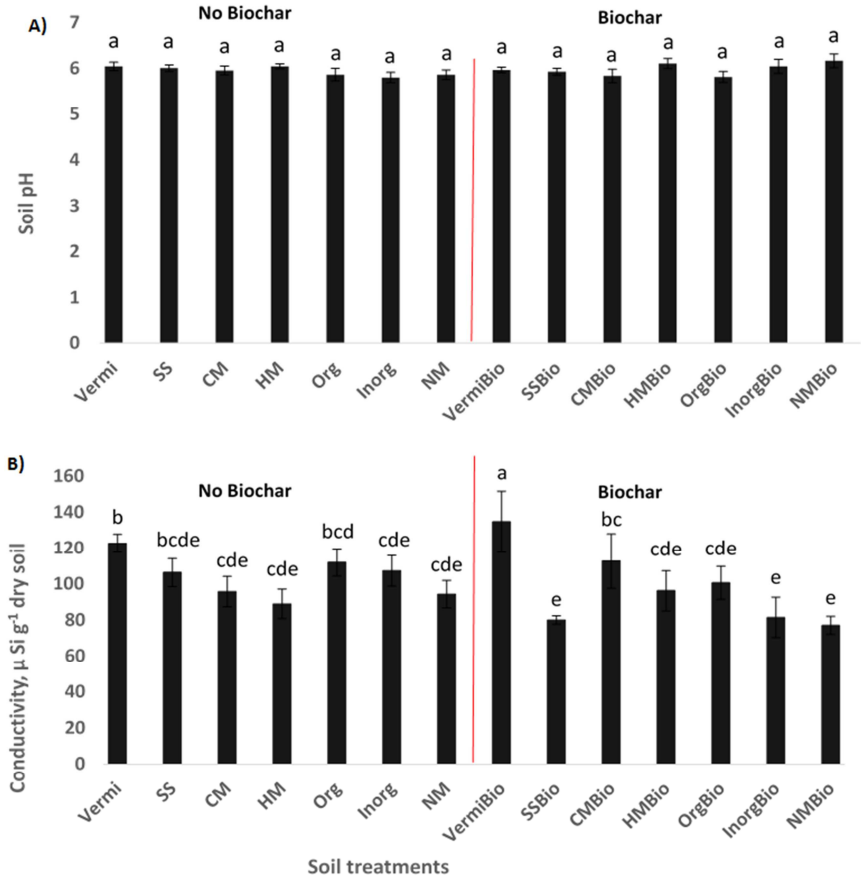

Figure 4. *Impact of soil amendments (SA) and SA mixed with biochar on soil pH (A) and conductivity (B) in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars ± standard deviation accompanied by different letter (s) indicate significant differences (P≤0.05) using Duncan's multiple range test.*

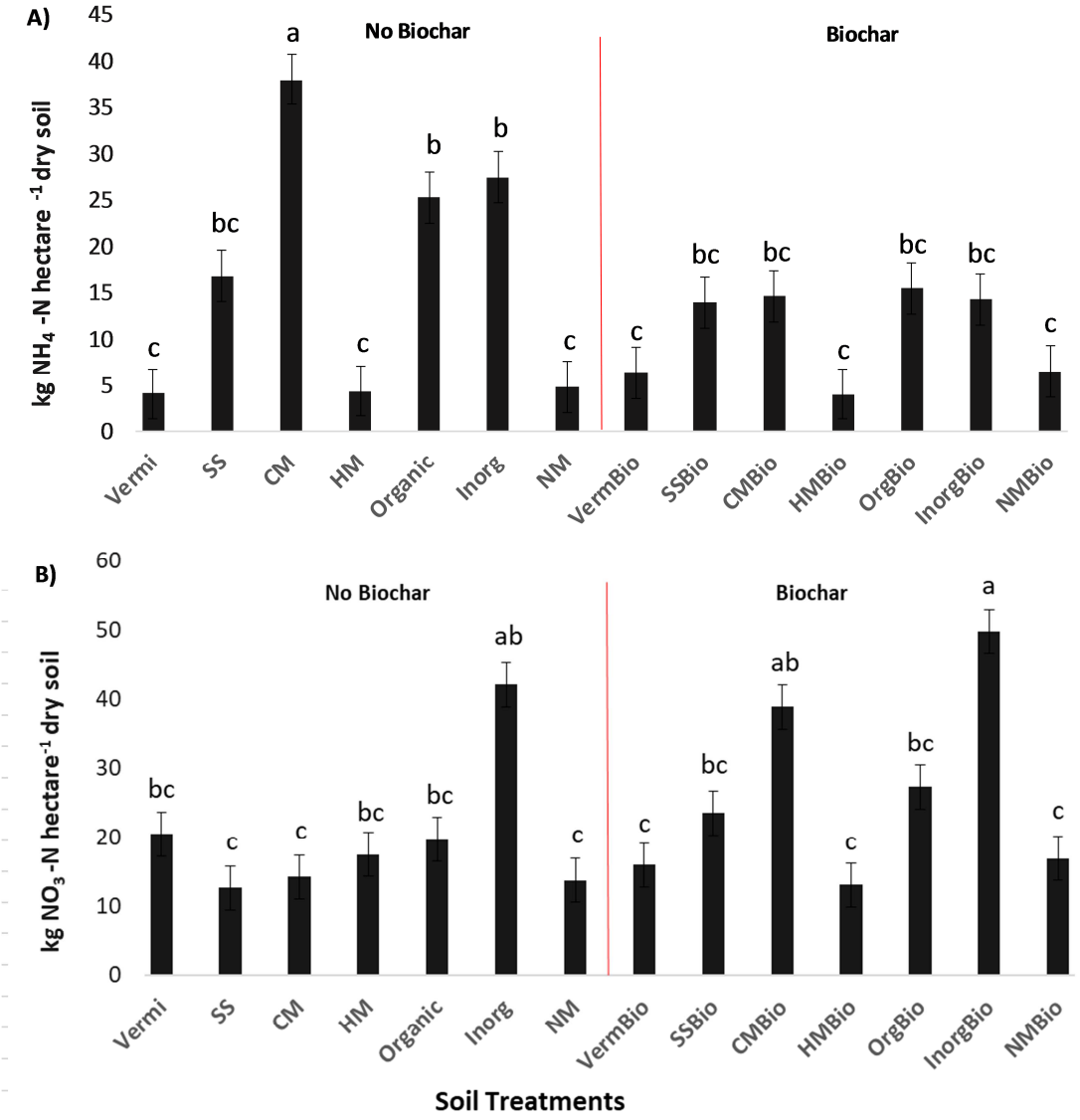

Figure 5. *Impact of soil amendments (SA) and SA mixed with biochar on soil ammonia (A) and nitrates (B) concentration in the rhizosphere of field-grown eggplants. Statistical comparisons were carried out among soil treatments. Bars ± standard deviation accompanied by different letter (s) indicate significant differences (P≤0.05) using Duncan's multiple range test.*

Application of different inorganic fertilizers and animal manures is a major source of ammonia (NH_3 -N) emission. The rate and total amount of NH_3 emission are related to different parameters such as, climatic conditions, soil characteristics and type of fertilizer [66]. Cameron et al. [67] reported that extensive N fertilizer application to agricultural soils increased N leaching into the soil and contaminated surface and groundwaters that may also deplete soil fertility and cause adverse impacts on environmental and human health. It is therefore important to develop and implement effective agricultural management practices to reduce N leaching. Accordingly, slow release of NO_3-N by biochar addition might prevent NO_3-N leaching [68] and provide NO_3-N to plants over a long period of time. Biochar has been shown to increase the potential for N retention in agricultural soils. Slow release of NO_3-N by charged organic matter used as a soil amendment, such as biochar was suggested as potential mechanism of nutrient slow release and delivery to plants [69].

Soil amended with CM increased NH_4-N concentration compared to all other amendments tested in this investigation (Figure 5A). Biochar added to CM (CMBio) increased NO_3-N by 2.7 times (Figure 5B). Other than that, no significant differences were found when biochar was added to other amendments. CM was superior in increasing NH_4-N concentration among all other amendments tested. No significant differences were found between Org and Inorg fertilizers even after the addition of biochar. Organic amendments like animal manures, good agricultural practices like cover crops, nutrient management, no-tillage techniques, and mulching can effectively be a major C sink that enhance agricultural sustainability [70]. Results of this investigation also revealed that Inorg and biochar added to Inorg fertilizers (InorgBio) significantly increased NO_3-N concentration compared to NM native soil (control treatment) (Figure 5B).

4. Conclusion

Municipal sewage sludge, horse manure, chicken manure, vermicompost, commercial organic and inorganic fertilizers amended with biochar were investigated for potential impact on soil enzymes (urease, invertase, acid and alkaline phosphatase) activity. Results revealed that the addition of vermicompost amended with biochar significantly (P<0.05) increased the activities of urease and invertase activity by 47 and 45%, respectively compared to the control treatment (no-mulch NM native soil). Soil acid phosphatase activity was reduced by addition of commercial inorganic fertilizer (Southern State NPK) compared to soil amended with commercial organic fertilizer (Nature Safe NPK). Whereas no significant differences were found in soil alkaline phosphatase activity between organic and inorganic fertilizers. No significant changes were detected in soil pH after the addition of soil amendments. Soil electrical conductivity fluctuated among the different soil amendments and was greater in vermicompost mixed with biochar compared to the control treatment (NM soil). It is important to mention that the organic matter loss during biochar preparation through the pyrolysis process contributes to an increase in the concentration of heavy metals in biochar. The influence of biochar on soil enzymes (urease, invertase, and phosphatase) has been found negative, positive, or insignificant. Potential metals accumulation in edible plants grown in soil amended with biochar and/or other soil amendments should be monitored and tested prior to large-scale application in agricultural production systems. Our future objectives will be focused on testing more than one type of biochar prepared from different sources and combine more than one type of animal manures to investigate their potential impact on total soil enzymes activity.

Authors Contributions

G. F. A. designed the study and conducted the laboratory soil analysis and wrote the manuscript. E. T. T. and D. S. S. assisted in the field and laboratory analysis and M. H. D. conducted the statistical analysis.

Conflicts of Interest

The authors declare that they have no competing interests**.**

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