

Review Article

Effect of Bone Char Application on Soil Quality, Soil Enzyme and in Enhancing Crop Yield in Agriculture: A Review

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Abstract

Soil quality, in contrast to air or water, exhibits a heightened level of heterogeneity and necessitates closer examination due to its impact on the well-being of flora, fauna, and human beings. Organic carbon is considered a fundamental indicator of soil quality, as it plays a significant role in strategies aimed at mitigating climate change. The generation of bone char arises from a thermochemical conversion process involving defatted bones. Specific attention is focused on the solubility of P compounds, which serves to classify bone chars as potential slow-release P fertilizers. The introduction of P into the soil can be enhanced through an "internal activation" process facilitated by the adsorption of reduced S compounds. Additional properties of agronomic significance originate from the porosity of bone char, which promotes water retention and provides a habitat function for soil microorganisms. The evaluation of soil quality has been a longstanding practice, involving an examination of physical and chemical characteristics such as pH, nitrogen levels, soil organic carbon, bulk density, accessible water, aggregate stability, particle size distribution, and soil structure. Recently, the concept of soil quality has been expanded to encompass the notion of soil health, which is perceived as a finite, non-renewable resource that undergoes constant change. Research also demonstrates the crucial role of soil biota in the assessment of soil quality, as they exhibit rapid responsiveness to disturbances. Animal bones undergo a process of defatting, degelatinization, and subsequent incineration at temperatures ranging from 600-800 °C to produce bone char (BC). Reports indicate that typical BC contains 152 g P kg⁻¹, 280 g Ca kg⁻¹, and 6.5 g Mg kg⁻¹, with carbon content typically falling below 100 g kg⁻¹. The solubility of bone char in the soil depends on factors such as pH and the soil's capacity to absorb P, situating it within the range between rock phosphate and triple super phosphate (TSP). The application of bone char to the soil can enhance soil health, resulting in increased crop yield and improved quality.

Keywords

Bone Char, Soil Microbes, Solubility, Soil Health, Phosphorus, Nutrients

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1. Introduction

As to Hubbard et al.'s study, there are three elements that make up environmental quality: air, water, and soil [28]. Definition of soil quality indicators are much broader and includes things like "the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health" [16, 17].

In fact, soil quality is more varied than that of air or water due to the fact that soils have a wider range of applications in addition to having solid, liquid, and gaseous phases. A type of soil's ability to function in an ecosystem; to sustain human health, the urban environment, protect nature and mitigating the climate change is termed as soil quality. Methods for investigating soil quality are accessible: (1) Intrinsic soil quality, which is contingent upon the processes involved in soil formation and represents the complete potential of the soil to perform a specific task influenced by management; and (2) Dynamic soil quality, which necessitates the integration of three primary components to attain efficiency and equilibrium: environmental quality, sustainable biological productivity, and the well-being of animals and plants. According to [1] the selection of appropriate soil parameters is imperative for evaluating soil quality and illustrating the condition of the soil.

The implied consequences of soil deterioration in soil health is the loss of essential functions of soil, such as providing physical support, water and nutrient necessary for terrestrial plant growth, controlling water flow in the environment and removing harmful contaminants through physical, chemical and biological processes. These functions essentially act as an environmental buffer [2]. The sustainability of agriculture, environmental and soil quality, all have an impact on human, plant and animal health [3, 4].

For numerous years, meat and bone meal (MBM) has been utilized in animal feed as an excellent source of minerals and proteins [5]. The utilization of MBM for animal feed was prohibited in the European Union in 2000 due to presence of transmissible spongiform encephalopathies [5]. Furthermore the inclusion of various organic chemicals amino acids, in MBM could potentially enhance the C/N ratio stimulate microbial activity, affect the sequence of microbial community during composting and ultimately influence the composting process. In comparison to animal manure, MBM presents a lower probability of spreading antibiotic resistance genes [6].

Since various microbes synthesize different enzymes to hydrolyze lignocellulose, it is beneficial to uphold a substantial microbial assortment and efficacy for degradation of asparagus straw biomass [7]. The low-cost by-product meat and bone meal, endowed with intricate organic compounds that provide a range of substances for microbial cultivation. Moreover, its composition comprises nitrogen (N), phosphorus (P), and potassium (K) with slower solubility rate

compared to their facile dissolution, thereby rendering it an exceptional selection for invigorating microbial activity [8, 9].

2. Significance of Review

The attribute of bone char exhibit a wide spectrum of variations, contingent up on the characteristics of source materials and pyrolysis conditions which foster the retention of water and serve as a habitat for soil microorganisms.

A novel and sustainable approach to mitigate soil degradation and decrease in agricultural productivity necessitates a paradigm shift in thinking and utilization of organic waste management techniques [10]. In order to fulfill future demands pertaining to soil health and the environment, methods such as nutrient recycling and other approaches aimed at enhancing soil quality with utilization of diverse organic waste materials are currently being explored. Among these, bone char stands out as a particular promising source that has the potential to safeguard the soil quality.

By repurposing animal bone waste, which would otherwise give rise to environmental and health concerns, one can effectively address the gap in soil health and nutrient related issues, thereby facilitating an increase in crop yield.

The aim of this review was to examine the impact of bone char on the improvement of soil quality and the availability of essential nutrients for crops. Additionally, it sought to evaluate the effectiveness of bone char amended soil compared to un-amended soil in terms of crop yield and the abundance and distribution of soil microorganisms and enzymes.

2.1. Bone Char

It is obtained from a Latin term "carbo animals," referring to a permeable, dark, grainy substance formed by the charred remains of animal bones. The constitution of this substance varies depending on the production method, primarily consisting of tricalcium phosphate (known as hydroxyl apatite) at a proportion ranging from 57% to 80%, accompanied by calcium carbonate at 6% to 10%, and carbon at 7% to 10% (Fawell et al., 2006).



Figure 1. Bone char with different size.

2.2. Different Size of Bone Char

Chowdhury, R. B. *et al.* [11] Found that charcoal, which is produced by heating biomass without oxygen, has numerous positive effects on the characteristics of soil. Char-derived carbon (C) is present in significant levels in some of the world's most productive soils, including chernozems [12], several anthrosols [1] and the renowned terra preta in Amazonia region [13]. Variations in reaction temperature, heating rates, and feedstock and vapour residence periods result lead to formation of pyrolysis products with distinct properties. These variations manifests in the different type of pyrolysis process that are currently available [14]. In order to convert wood in to charcoal, a slow pyrolysis technique is employed. To make charcoal from wood, slow pyrolysis at low r involving low reaction temperatures (ranging from 200 to 400 °C) and extend residence time for both vapors and feedstock, which may span several times or even days [14]. The primary objective of fast pyrolysis is to maximize the production of pyrolysis liquid from wood and other lignocellulosic materials, which process the potential to serve as substitute for fossil fuels. To this end, fine particle feedstock is rapidly heated to 500 °C and subjected to a very brief residence period. As a consequence, reduced levels of char and gases are generated [15].

Animal bone chips are subjected to the process of defatting, degelatinization, and subsequent pyrolysis at temperature ranging from 600 to 800 °C to yield BC [16]. BC typically exhibit elemental concentrations of 152 g P kg⁻¹, 280 g Ca kg⁻¹, and 6.5 g Mg kg⁻¹ as reported in [17]. It is noteworthy that carbon content levels lower than 100 g kg⁻¹ are commonly observed [18, 19]. It has been highlighted in literature that the solubility of BC is contingent upon various factors, including pH and the soils capacity to sorb P. furthermore, it is situated in position between the rock phosphate and triple super phosphate (TSP) range [20], in an effort to enhance the solubility of P, a surface modified variant of BC, enriched with the addition of sulfur (S), has been formulated and referred as BC^{plus}.

The initiation of incubation test and pot experiment revealed an improvement in solubility [21]. Furthermore, recent field investigations have indicated the potential of BC plus to elevate the concentration of accessible phosphorus in soils with initially low levels of phosphorus [22]. Given that phosphate (P), calcium (Ca) and magnesium (Mg) are crucial component of plant nutrition and are abundant in bones, chars produced through the pyrolysis of bone materials may possess a greater value in terms of soil fertility. Bone char has historically been utilized in the production of paints under the name “black bone” and as a filtering material for the purification of sugar. The porosity and crystal structure of bone chars are influenced by factors such as mineral content three [23], animal species and age [24], and pyrolysis conditions [25]. The crystallinity of bone can be enhanced through an increase in mineral maturity and mineralization during the growth of an animal [26, 24].

2.3. Soil Quality Indicators

Soil quality has traditionally been evaluated predominantly based on its physical and chemical attributes. The most commonly employed parameters for assessing soil quality pertain to its properties, including pH, total nitrogen, and soil organic carbon as well as its physical characteristics such as bulk density, available water, aggregate stability, soil structure, and particle size distribution. As stated by [27], the initial definition of soil quality is subject to constant modification and has involved into the concept of “soil health,” which portrays soil as a finite, non-renewable, and dynamic resource. Although some scholars argue for a distinction between these terms, both concepts are considered to be synonymous and are widely used interchangeably [27]. To gain a comprehensive understanding of soil quality, various authors emphasized the importance of indicators of diverse types (physical, chemical and biological) over the past three decades [28, 29]. More recently, additional factors such as social wellbeing and economic dimensions have been incorporated into this approach to develop appropriate soil indicators for sustainable soil management [30].

Soil organic carbon has long been considered as a prominent indicator of soil quality, with extensive connection to various soil functions and its crucial role as a primary carbon sink in terrestrial ecosystem [31]. This makes it crucial in strategies aimed at addressing climate change [32]. Numerous local, regional and international investigations have focused on studying soil organic carbon as a marker of soil recovery or deterioration [33].

The soil's biological component plays a significant role in assessment of soil quality as confirmed by an increasing body of research [33]. As stated [34], the soil biota assumes responsibility for various aspects of the soil ecosystem and can respond rapidly to environmental changes, particularly following disturbances or recovery of the ecosystem. Additional studies suggested that when variables display a high level of association, the utilization of numerous variables can result in redundancy [35].

The modification of soil microorganisms and enzymes activities is observed when organic or inorganic amendments are applied to the soil [5]. Soil enzymes are utilized as indicator of soil health and are typically employed to detect rapid changes in soil microorganism activity in response to the addition of biochar. The supplementation of biochar with nutrient enhances microbial activity, consequently leading to an increase in the activity of specific enzyme such as urease, phosphatase and beta-glucosidase. Urease which plays a crucial role in the nitrogen cycle, relies on carbon and nitrogen as a source of energy and electron transfer due to the associated microbial community [36]. The introduction of biochar derived from s significantly to the availability of dissolved organic carbon (DOC) and nitrogen, thereby enhancing urease activity [37]. Similarly, the addition of carbonized bone (CB) also improves the availability of phosphorus, which in turn enhances the activity of phosphatase- linked microbial community availability

of P improved by CB addition also enhanced the phosphatase linked microbial community, leading to an improvement in activity of acid phosphatase (APA) in the soil. The results from comprehensive correlation analysis (CCA) also indicate that the changes in the soil nutrient status induced by biochar are the primary factors driving soil enzyme activity.

Improvement in soil properties can enhance the activity of BGA due to its sensitivity to soil pH and carbon content [38]. However, the production of carbon black (CB) at high temperature has been found to have negative effect on soil microbial communities, resulting in decreased activity of specific enzymes like dehydrogenase [39]. The adverse impact of CB on soil microbiology has also been documented in other studies. The

measurement of dehydrogenase activity (DHA) provides an indication of the overall activities of soil microorganisms [40].

2.4. Bone Char Effect on Soil Physicochemical Properties

Animal bones serve as an exceptional reservoir of phosphorus (P) due to their notably low levels of heavy metals and elevated of P concentrations, in addition to key elements for plant nutrition such as Ca, Mg [41]. In study by [20], the significant impact of soil pH on the release of P from bone char (BC) was demonstrated.

Table 1. Selected physicochemical properties of the soil and bone char used in the study.

Properties	Unit	Soil	Biochar	
			SBL	SBH
EC	($\mu\text{S cm}^{-1}$)	5.39	1420	1874
pH		8.2	8.82	10.55
Soil texture		Sandy loam	–	–
Surface area	(m^2g^{-1})	–	90.8	103
SSA (micropores)	(m^2g^{-1})		0.76	1.29
SSA (external)	(m^2g^{-1})		98.5	104.3
Average pore diameter	(nm)		11.34	0.257
Total pore volume	(cm^3g^{-1})		11.50	0.296
CEC	$\text{cmol}_c\text{kg}^{-1}$	24.6	–	–
SOM	g kg^{-1}	14.9		
DOC	mg kg^{-1}	22.5	434	118
C	%		11.0	9.4
H	%			
N	%	0.122	2.0	1.8
O	%		29.2	34.6
S	%		13.8	12.6
K	g kg^{-1}		30	27.5
Ca	g kg^{-1}		231	288.9
Mg	g kg^{-1}		8.9	10.1
TP	%	0.08	10.6	12.8
Metals	(mg kg^{-1})			
Bioavailable Zn		474	23.8	23.5
Total Zn		981	29.33	28.09
Bioavailable Cd		6.71	ND	0.1
Total Cd		18.2	ND	ND

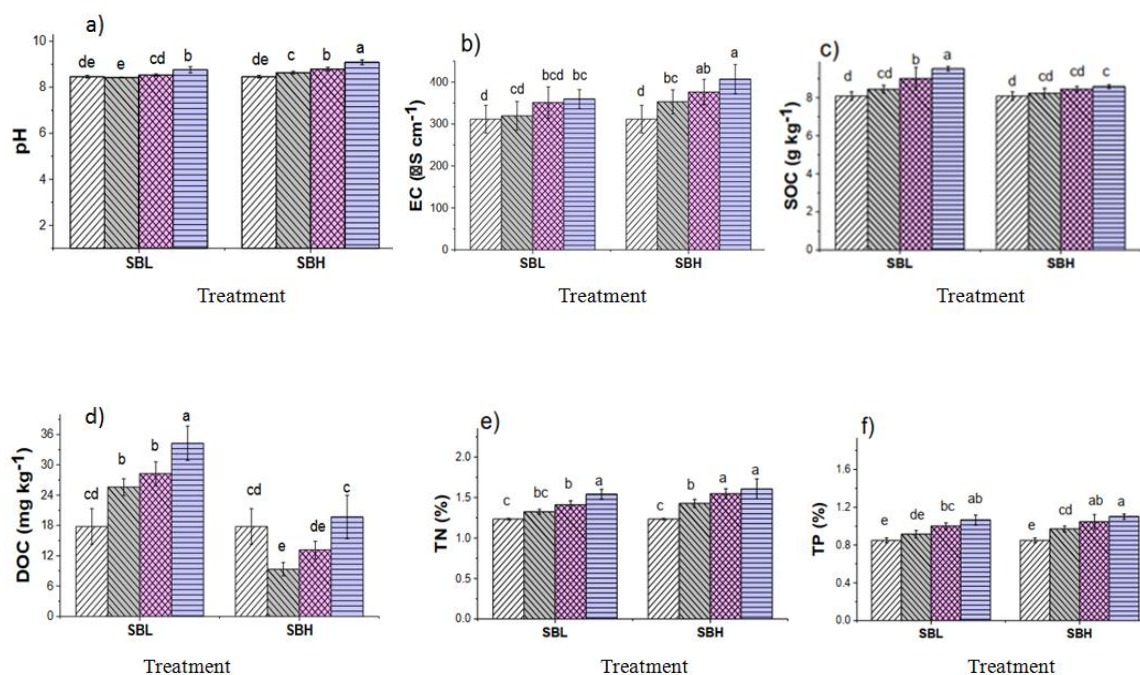


Figure 2. Sheep bone-derived biochar-induced changes in soil chemical properties. Different letters within each parameter indicate significant differences at $P \leq 0.05$.

The utilization of BC prompted an increase in the soil pH. The introduction of SBL₁₀ led to a notable 8.9% elevation in the levels of DOC in comparison to the control. Moreover, SBH resulted in a simultaneous increase of 31% in electrical conductivity (EC), 0.62 units in total nitrogen (TN), and 29% in total phosphorus (TP) as compared to the control. The application of higher pyrolysis temperature rendered the SBH alkaline, thereby causing an elevation in the soil pH subsequent to its placement. The quantity of DOC present in the soil substantially influences the level of metals, and the inclusion of SB reduced the DOC content, possibly leading to greater retention of metals by the BC [42]. It is widely acknowledged that the carboxylic and phenolic groups, alongside biodegradable DOC, can significantly impact the extent to which a plant can absorb trace metals (Beesley et al., 2010).

Secondly, an increase in the pH of the soil may have also played a role in the heightened sorption of metals on the biochar surface [43]. A higher pH level leads to a reduction in the presence of free hydrated metal cations and subsequently affects their ability to move within the soil. Consequently, this has an impact on the sorption and precipitation of metals (and metalloids) species that carry a positive charge. Moreover, the pH of the solution also affects the affinity of the biochar surface. The introduction of Ca and Mg ions from SB, which contributes to an elevation of base saturation and soil pH, could have further contributed to the observed increase in soil pH. Additionally, the reaction between the hydroxide ions and soil CO_2 generates more alkalinity and subsequently results in the production of secondary HCO_3^- .

Table 2. Characteristics of soil, bone char and biochar-based amendments used in this study.

Treatments	pH	C %	N	Mehlich-P mg/kg	K	Ca	Mg	S	CEC cmol _c /kg
Biochar	9.95	67.34	1.46	497.54	10549.56	1485.86	603.69	835.14	17.05
Bone char	6.99	7.28	1.47	5088.80	5380.60	23683.11	2363.02	102.67	n.a
Compost	7.6	26.25	2.19	4310.07	5567.02	8948.88	1967.10	219.78	50.94
Soil	5.08	3.13	0.28	14.5	646.64	1202.84	204.17	11.48	41.26

Before application of the land, samples of bone char, biochar, and compost were gathered. The total carbon content was measured using the loss on ignition method while the total nitrogen content was determined through dry combustion. Additionally, a solution consisting of 0.2 N acetic acid; 0.25 N NH_4NO_3 ; 0.015 NH_4F ; 0.013 N HNO_3 ; and 0.001 M EDTA was utilized to measure the concentration of a primary nutrients (phosphorus and potassium), secondary nutrients (calcium, magnesium and sulfur), iron and aluminum at a ratio of 1:10 (w/v) [44].

To incorporate soil amendments, ploughing depth was utilized and the land was subsequently prepared using a hand hoe. It was expected that the treatment effect would be limited to a

shallow depth. In May 2020, soil samples were collected from the treated areas at the depth of 10 cm. these samples were air dried and crumbled before being subjected to sorption/desorption experiments and chemical analysis. The soil pH was determined using pH meter with soil to water ratio of 1:2.5. the carbon content of the soil was assessed using the loss on ignition method, nitrogen content was measured through dry combustion, and phosphorus content was determined using the Bray II extraction solution (0.025 M HCl ; 0.03 M NH_4F) at a soil-to-solution ratio of 1:10. For the evaluation of cation exchange capacity (CEC) 1 N ammonium acetate was employed with a pH of 7.0 [45].

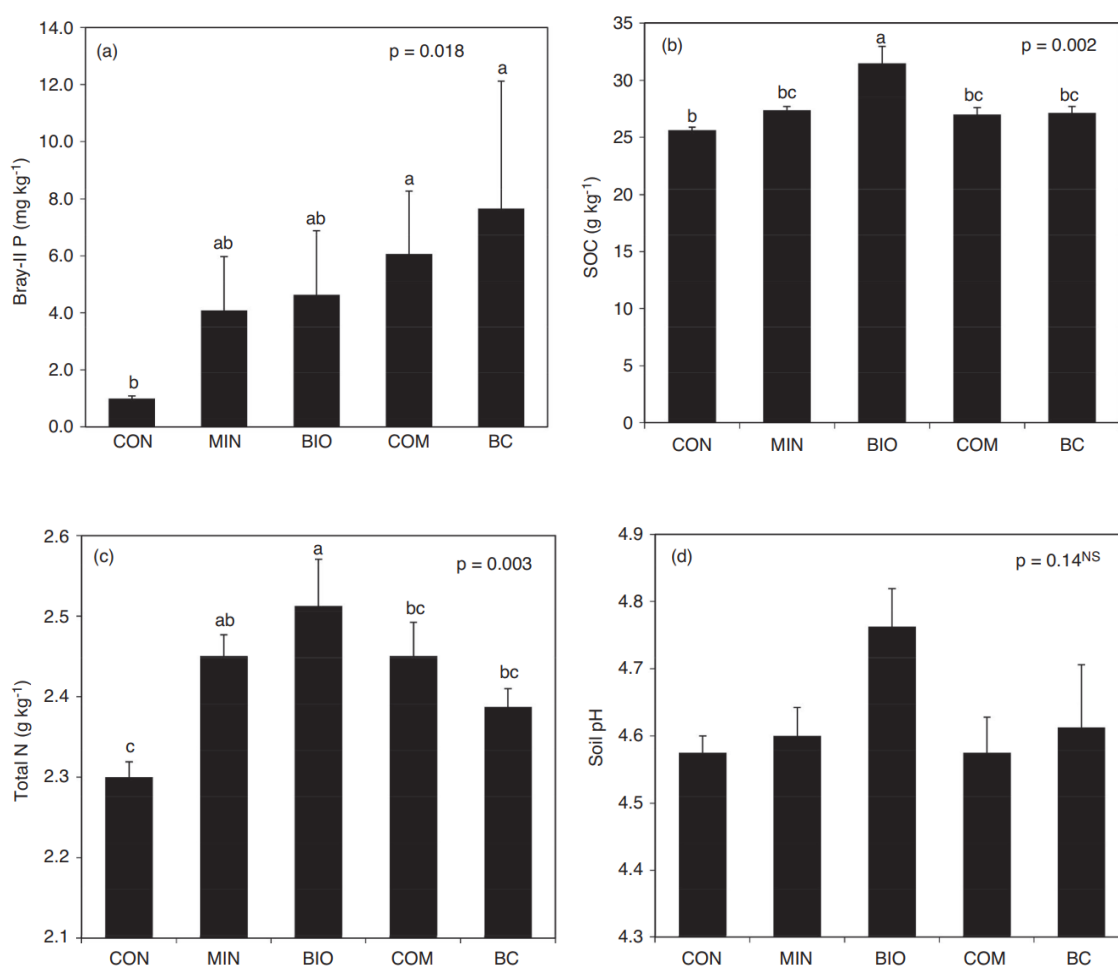


Figure 3. Soil properties after bone char and biochar-based soil amendment addition for eight years (means \pm SEM). (a) Plant available P, (b) soil organic carbon, (c) total nitrogen and (d) soil pH. $p < .05$.

After a consistent period of eight years, the utilization of soil amendment containing BC and biochar resulted in an increase in the availability of phosphorus to plants, soil organic carbon (SOC), total nitrogen, and C/N ratio observed in soil amended with biochar can be attributed to the input of organic matter from biomass (i.e., roots and shoots) as well as the biochar itself. In comparison to the control, the presence of BC and biochar in

the soil led to seven fold and five-fold increase in plant available phosphorus, respectively (as shown in Figure 2a). previous studies have already indicated that the liming effect of biochar enhances the accessibility of phosphorus to plants [46, 47]. However, the differences in plant available P across treatments cannot be attributed to liming effects, as none of the treatments had significant impact on soil pH (as illustrated in figure 2b).

Instead, the highest level of available plant available P can be explained by the substantial P content present in the soil amendments, such as compost, BC, and biochar. Among the soil amendments, BC exhibits the highest concentration of Mehlich P, reaching 5089 mg/kg (as shown in Table 2) (Figure 3a). the phosphorus availability achieved through the utilization of bone char treated soil was comparable to that obtained from compost and mineral fertilizer, affirming the potential of abattoir waste valorization in meeting future phosphorus fertilizer demands and mitigating the environmental consequences associated with mineral waste production [48]. Furthermore, similar findings of P solubilization and increased bacterial abundance in bone char amended soils were reported by [49], which aligns with the outcome of the study. The recovery of P from slaughterhouse waste is of paramount importance, particularly in developing nations where waste poses a significant environmental threat and smallholder farmers are unable to

afford mineral fertilizers.

As far as the authors are cognizant, this investigation represents the inaugural attempt to probe the ramifications of protractedly implementing BC on plant accessible P content, crop yield, and P adsorption-desorption procedures.

No significant correlation between P adsorption and soil attributes (pH, SOC, N, C/N ratio, and accessible P) was observed, implying the excessive nutrient content and liming are improbable causes of the substandard P adsorption under BC and biochar treatments. The primary mechanism accounting for the diminished P adsorption beneath the biochar and BC treated soils is likely the modification of functional groups on biochar surfaces. Consequently, further research is recommended to ascertain whether the surface functional groups of biochar undergo alteration with time, thereby impacting the P adsorption-desorption procedures.

Table 3. Selected soil chemical characteristics and bioavailable Zn and Cd influenced by bone-biochar application (means \pm SD, $n = 3$).

Treatments	EC (dS m ⁻¹)	pH	DOC mg kg ⁻¹	SOC g kg ⁻¹	TN	TP mg kg ⁻¹	DTPA-Zn	DTPA-Cd	P/Zn ratio
CBL-0	0.32 \pm 0.02 ^d	8.46 ^{cd}	23 \pm 2 ^c	8.03 \pm 0.2 ^d	1.21 \pm 0.01 ^d	803 \pm 25 ^b	474 \pm 15 ^a	7.1 \pm 15 ^a	1.70 \pm 0.09 ^c
CBL-2.5	0.32 \pm 0.01 ^d	8.55 ^b	30 \pm 3 ^b	8.28 \pm 0.2 ^{cd}	1.37 \pm 0.05 ^{bc}	994 \pm 31 ^a	402 \pm 11 ^{cd}	5.8 \pm 0.1 ^c	2.47 \pm 0.11 ^b
CBL-5	0.37 \pm 0.02 ^{bc}	8.50 ^{bc}	33 \pm 3 ^b	8.84 \pm 0.6 ^b	1.49 \pm 0.06 ^{ab}	1010 \pm 108 ^a	384 \pm 23 ^d	5.4 \pm 0.2 ^d	2.64 \pm 0.36 ^b
CBL-10	0.58 \pm 0.03 ^a	8.39 ^d	40 \pm 3 ^a	9.36 \pm 0.1 ^a	1.52 \pm 0.18 ^a	1023 \pm 27 ^a	283 \pm 13 ^e	4.7 \pm 0.2 ^e	3.61 \pm 0.10 ^a
CBH-0	0.32 \pm 0.02 ^d	8.46 ^{cd}	23 \pm 2 ^c	8.03 \pm 0.2 ^d	1.21 \pm 0.01 ^d	803 \pm 25 ^b	474 \pm 15 ^a	7.1 \pm 0.2 ^a	1.70 \pm 0.09 ^c
CBH-2.5	0.33 \pm 0.01 ^d	8.57 ^b	9 \pm 2 ^e	8.12 \pm 0.3 ^{cd}	1.28 \pm 0.03 ^{cd}	827 \pm 40 ^b	441 \pm 27 ^{ab}	6.2 \pm 0.3 ^b	1.88 \pm 0.17 ^c
CBH-5	0.36 \pm 0.01 ^c	8.57 ^b	10 \pm 2 ^e	8.33 \pm 0.1 ^{cd}	1.37 \pm 0.05 ^{bc}	1024 \pm 37 ^a	426 \pm 24 ^{bc}	6.0 \pm 0.2 ^{bc}	2.41 \pm 0.16 ^b
CBH-10	0.40 \pm 0.02 ^b	8.78 ^a	16 \pm 3 ^d	8.47 \pm 0.1 ^b	1.50 \pm 0.06 ^a	1027 \pm 18 ^a	381 \pm 18 ^d	5.1 \pm 0.2 ^{de}	2.70 \pm 0.15 ^b

Interactive effects of CBL and CBH resulted in significant alteration in the physical and chemical properties of the soil (Table 1). In comparison to the control, the application of CB led to an increase in soil electrical conductivity and pH. The CBL10 treatment was associated with higher soil EC values of 0.58dS/m, while the CBH10 treatment was linked to a higher soil pH value of 8.7. The application of CBL resulted in noteworthy increase in soil DOC and SOC content, whereas, the CBH treatment led to a reduction in DOC content. Overall, the CBH10 treatment exhibited an 83% increase in soil EC, a 74% increase in total DOC, a 16% increase in SOC, a 26% increase in total nitrogen (TN) and 27% increase in TP content, as compared to the un-amended control. However, the pyrolysis temperature did not have an impact on soil TN or TP contents, additionally, the application of CBL at a rate of 10% resulted in a significant increment of 112% in the phosphorus

to zinc (P/Zn) ratio.

2.5. Bone Char Effect on Soil Microbes

Microorganisms inhabiting the soil possess considerable significance in various aspects such as ecosystem productivity, nutrient circulation and climate regulation [50]. The types and abundance of these microorganisms may differ depending on the location as they exhibit remarkable adaptability to diverse environmental conditions. Furthermore, the types and persistence of this microorganisms are directly related to the duration and quality of composting [51-53]. Research conducted by both [52, 53], has delved into the enhancement of composting efficiency by screening microbial populations during the mesophilic and thermophilic phases of composting. According to [54], the richness and diversity of soil microbial

communities play a fundamental role in governing pivotal functions within ecosystem, such as gaseous fluxes, nitrogen cycling, and decomposition of organic matter. Alteration in the composition of soil carbon (C) may result in significant imbalances in the relationship between microbial diversity and biomass. This connection is particularly susceptible to the impacts of climate change and intensified land use practices [55].

The introduction of SB resulted in significant alteration in the composition bacteria residing in the soil. Following addition of SBL 2.5, there was an increase in Proteobacteria, Gemmatimonadetes and Firmicutes, whereas their abundance decreased after SBL 10. Conversely, other bacterial groups such as Actinobacteria, Chloroflexi, Saccharibacteria, Parcu bacteria, Verrucomicrobia, Armatimonadetes, and Microgenomates exhibited an increase in abundance after SBL₁₀. However, the higher dose SBL10 resulted in a decline in a certain bacterial communities including Gemmatimonadetes, Acidobacteria, Nitrospirae, Cyanobacteria and Planctomycetes. In the case of SBH treated soil, Bacteroidetes, Saccharibacteria, Verrucomicrobia, cyanobacteria, Chlorobi and

Microgenomates all exhibited an increase in abundance after SBH10, while Actinobacteria, Gemmatimonadetes and Planctomycetes showed a decrease. Notably, some bacteria exhibited a greater increase after ABH compared to SBL, while others displayed a larger decrease after SBH than after SBL. The Proteobacteria community only experienced a decrease after SBL10.

The alteration of physical and chemical characteristics caused by introduction of biochar can have an impact on composition, abundance and function of bacteria [56, 57]. This phenomenon may attribute to the addition of DOC and nutrients by biochar, which can subsequently lead to an increase or decrease in microbial populations [58]. Furthermore, the porous structure and extensive surface area of biochar provide a favorable habitat for microorganisms, shielding them from predation by other organisms [59]. Additionally, the high water retention capacity of biochar contributes to the survival of sensitive bacteria during dry season/periods. The ability of biochar to retain certain molecules can also stimulate bacterial diversity and population size [60].

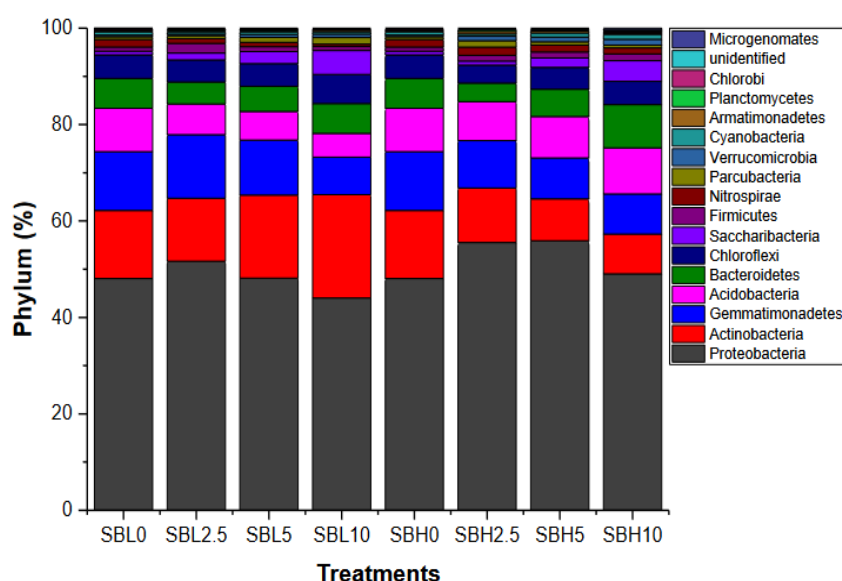


Figure 4. Effect of sheep bone char on the dominant bacterial phyla.

However, its impact to note that the effect of biochar on bacterial population and diversity are contingent upon factors such as biochar type, processing methods, duration of exposure to the soil and soil conditions [61, 62].

The addition of biochar to the soil resulted in an increase in pH and carbon content, which provide to be advantageous for Proteobacteria while not as beneficial for Actinobacteria. The utilization of high temperature biochar led to a reduction in the release of biodegradable DOC, consequently diminishing the abundance of Actinobacteria and restricting carbon losses.

Nevertheless, the elevated pH levels induced by the biochar were conducive for both Proteobacteria and Actinobacteria,

with this effect being more pronounced when the biochar was subjected to higher temperatures. Conversely, lower pH values were found to promote the growth of Acidobacteria [63].

Microbial communities are also affected by the rates of application the excessive dosage of low temperature biochar carries certain adverse effects, possibly linked to the toxic substances present in the biochar. Additionally, a high dosage of application enhances the soil's capacity for water retention, potentially impeding the establishment of certain community such as Gemmatimonadetes. In their study, the researchers [64] highlight that these communities thrive more in arid environments with a neutral pH.

Regarding *Firmicutes*, it is plausible that the presence of high levels of phosphorus could potentially facilitate the proliferation of these bacterial communities. Specifically, the increased abundance of *Firmicutes* in the forest soils can be attributed to escalated levels of phosphate resulting from the continuous application inorganic and organic fertilizers over several decades (Kuramae et al., 2012). As per the findings of [56], the phylum *Nitrospirae* is believed to encompass lineages responsible for nitrogen nitrification and overall nitrogen cycling, with *Nitrospira* one of its predominant genera. The decline in the prevalence of *Nitrospira* subsequent to the introduction of biochar may be attributed to the plausible mechanism of electrostatic and pH dependent adsorption of NO_3^- onto the surface [65].

2.6. Bone Char Effect on Soil Enzymes Activity

Soil enzymes, namely urease (UA), dehydrogenase (DA), β -glucosidase (BGA) and phosphatase (PA), play a crucial role in assessing the overall health of the soil. Notably, a statistically significant disparity ($P \leq 0.05$) in the activity of these soil enzymes was observed subsequent to the applica-

tion of SB (Figure 4). The application of SBL10 resulted in a notable increase of 33% in BGA activity, while the activity of DHA remained unaltered when compared to the control. Conversely, the application of SBH10 led to a substantial increase of 98% and 107% in UA and PA activities, respectively compared to the control. However, the activity of DHA and BGA decreased by 58% and 30%, respectively, following the SBH10 treatment in relation to the control. It is worth noting that the introduction of organic amendments into the soil has the potential to modify the microbial function and activity by providing supplementary nutrients and inducing changes in pH [66], as well as indirectly ensuring the availability of the other essential factors, such as water. The enzymes tested in this study are directly involved in the cycling of nutrients, specifically carbon, nitrogen and phosphorus and also serve as electron carriers in various metabolic pathways [36]. The substantial content of C, N, and P in SB significantly enhanced the activities of UA, BGA, and PA. The reduced activity of DA and BGA observed after the SBH treatments may be attributed to the relatively low concentration of biodegradable DOC in the soil.

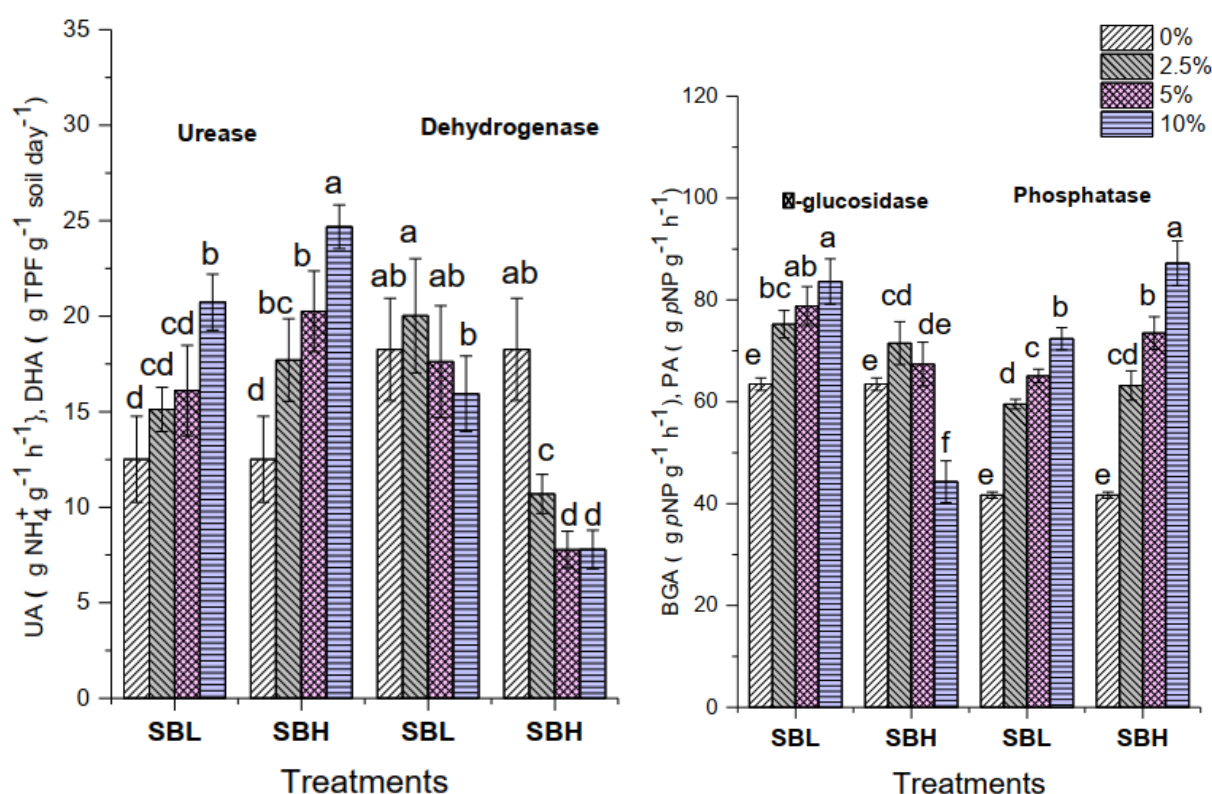


Figure 5. Effect of sheep bone-biochar on the soil enzyme activity.

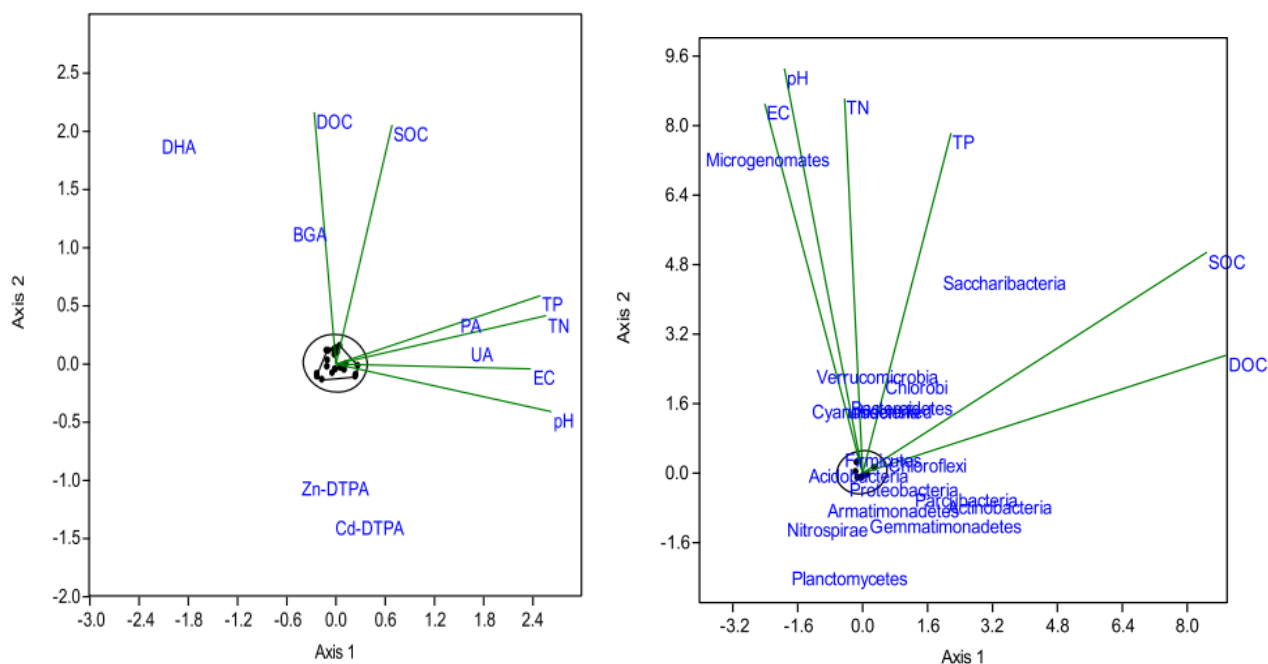


Figure 6. Correspondence analysis (CCA) of a) soil chemical properties with enzyme activity; b) soil variables c) soil chemical properties with bacterial phyla.

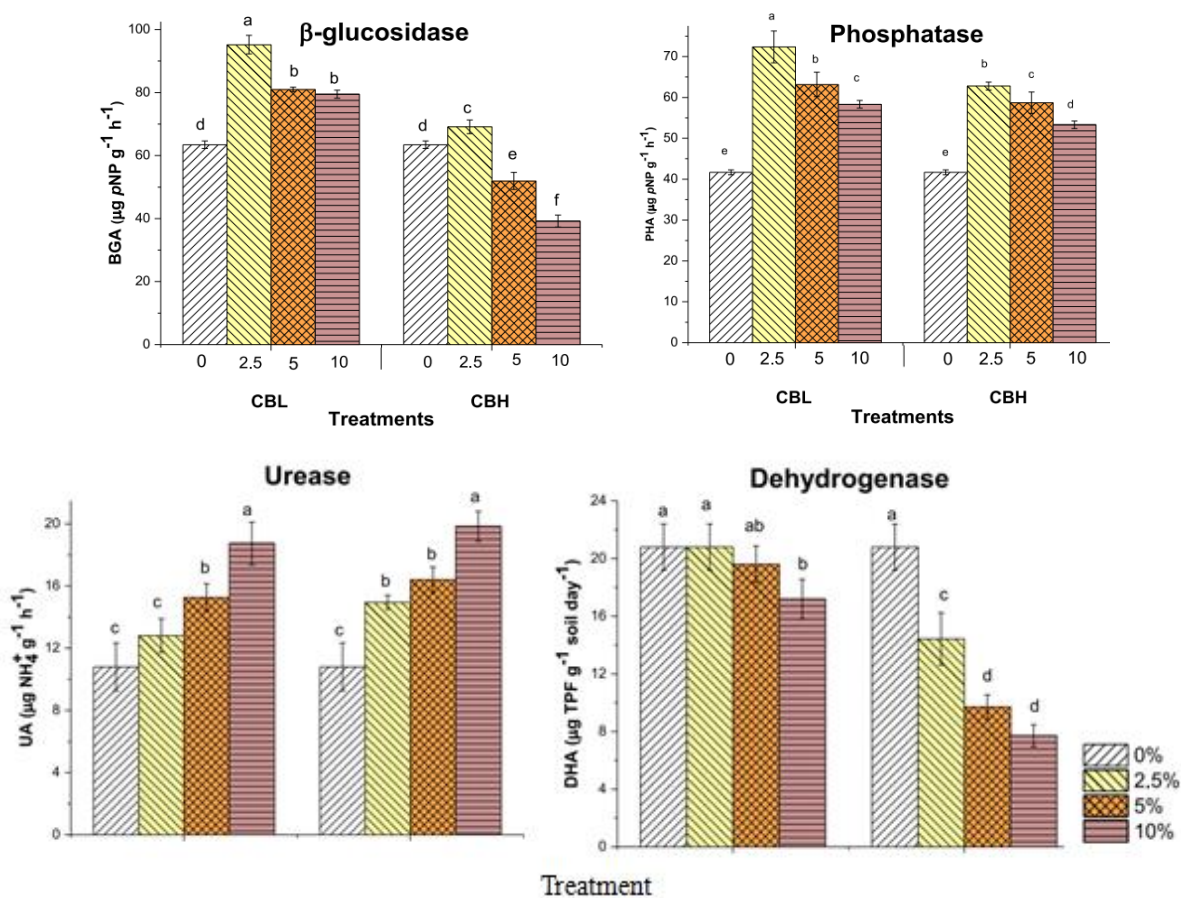


Figure 7. Effect of cow bone-biochar (CB) on soil enzyme activity.

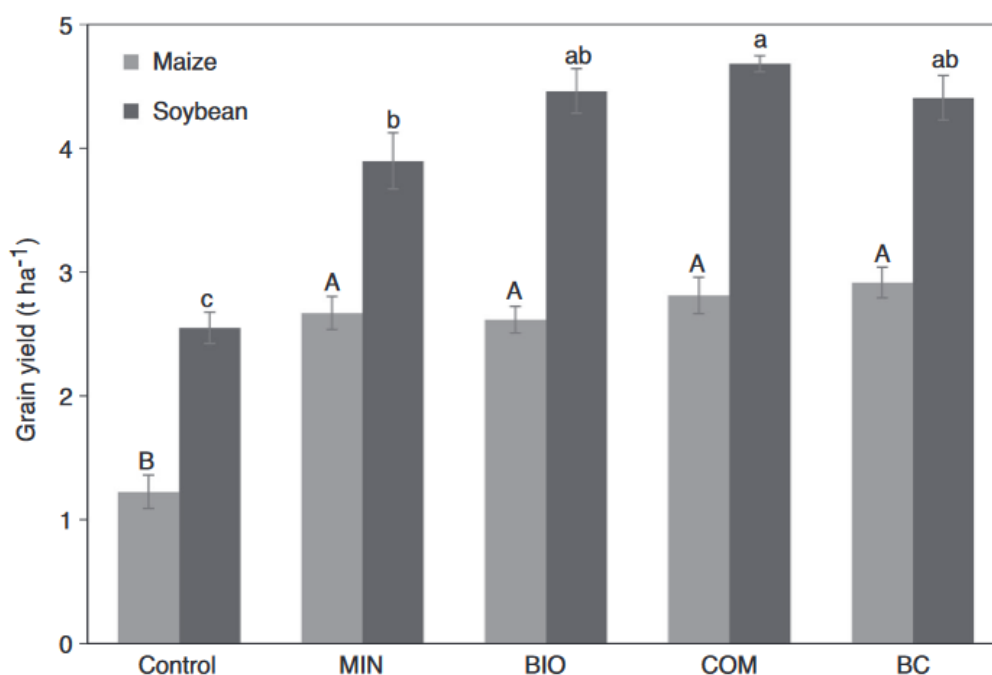
To gain a deeper comprehension of the reaction of enzyme activities to the application of SB, the utilization of corre-

spondence analysis was employed to demonstrate the response of soil enzyme activity and the concentration of bioavailable metals in conjunction with enhanced soil chemical (Figure 5a). The initial two axes account for a cumulative variance of 96.7%. Within axis-I, pH, EC, TN, and TP displayed the strongest correlation with UA and PA. Conversely, within axis-II, DOC and SOC exhibited the most robust correlations with DHA and BGA.

The inclusion of CB had a significant impact on soil enzyme activities ($P < 0.05$), and both the temperature of pyrolysis and quantity of CB applied yielded noticeable distinctions (Figure 6). When the rate of CB application was increased, UA activity exhibited improvement, regardless of the pyrolysis temperature. Conversely, DHA experienced a decrease with CBH and as the rate of CB application increased. The introduction 2.5% CB led to an increase in BGA and PHA activity, irrespective of the pyrolysis temperature, but this activity declined with greater amount of CB applied. The most significant increase in UA (88%) was observed with CBH10, followed by CBL-10 (77%), while DHA was reduced by (62%) in the case of CBH-10. The most substantial increase in BGA (51%) and PHA (71%) were observed with CBL-2.5, yet both BGA and PHA decreased with a higher quantity of CB applied, resulting in a 38% reduction in BGA after CBH-10.

2.7. Effect Bone Char on Crop Yield

Over the span of eight years, measurements were taken to determine the grain yields of soybeans and maize. The findings were presented in Figure 7. The introduction of biochar and bone char resulted in an increase in average yields of 1.4 Mg ha⁻¹ and 1.7 Mg ha⁻¹ and 1.8 Mg ha⁻¹ and 1.9 Mg ha⁻¹, respectively, for soy beans and maize. These outcomes align closely with the findings of [67], which observed a 1.2 Mg ha⁻¹ increase in maize production in a sub-humid region of Kenya as a result of biochar use. However, the yield increase for soybean in this particular study was nearly three times higher than the previous one [67], indicating that the advantages of biochar may vary depending on the specific crop, local soil condition, and biochar feedstock. The enhanced yields under bone char and biochar treatments can be attributed to the higher concentration of nutrients, such as plant available P (as depicted Figure 3a), and cations, like Ca and Mg, obtained from bone char (as shown in Table 1; [49]). The slightly higher yield under biochar treatment in comparison to bone char treatment can be attributed to the non-nutrient benefits of biochar, including improved soil structure and increased water holding capacity [68].



Source: (Wakweya et al., 2022)

Figure 8. Average maize grain yield after bone char and biochar-based soil amendment addition for eight years (means \pm standard error of the mean). Different letters denote significant differences at $p < 0.05$; BC, bone char; BIO, coffee husk biochar; COM, compost; MIN, mineral fertilizer; NS, non-significance. Error bars indicate standard error of the mean ($n = 4$). The capital letters represent maize yield, and the small letters represent soya bean yield.

Table 4. Effect of sheep bone-derived biochar on the maize metals concentration and growth parameters.

Temp.	Treat- ment	Root Zn mg kg ⁻¹	Shoot Zn	Root Cd	Shoot Cd	SL	SFW	SDW	RL	RFW	RDW
						g kg ⁻¹					
500 °C	SBL ₀	593±22 ^a	258±9 ^a	7.27±0.5 ^a	1.28±0.09 ^a	65±5 ^d	23±1 ^d	11±1.0 ^e	7.1±0.51 ^d	5.0±0.3 ^e	3.1±0.2 ^d
	SBL _{2.5}	430±17 ^b	233±11 ^b	5.05±0.2 ^b	0.94±0.08 ^b	71±6 ^{cd}	26±6 ^{cd}	13.3±0.58 ^d	7.4±0.65 ^d	5.2±0.5 ^{de}	3.2±0.3 ^d
	SBL ₅	372±18 ^c	196±8 ^c	3.92±0.2 ^c	0.80±0.08 ^{bc}	73±4 ^{cd}	30±4 ^{bc}	14±11.0 ^{cd}	8.4±0.25 ^c	5.8±0.6 ^{cd}	3.6±0.3 ^{cd}
	SBL ₁₀	278±17 ^e	173±12 ^d	3.22±0.1 ^{de}	0.60±0.04 ^{de}	82±3 ^{ab}	32±3 ^b	15.5±0.87 ^{bc}	9.4±0.46 ^b	6.8±0.5 ^{ab}	4.1±0.3 ^{ab}
800 °C	SBH ₀	593±22 ^a	258±9 ^a	7.27±0.5 ^a	1.28±0.09 ^a	65±5 ^d	23±1 ^d	11±1.0 ^e	7.1±0.51 ^d	5.0±0.3 ^e	3.1±0.2 ^d
	SBH _{2.5}	409±18 ^b	182±12 ^{cd}	4.69±0.3 ^b	0.81±0.08 ^{bc}	75±2 ^{bc}	29±3 ^{bc}	14.3±0.58 ^{cd}	8.4±0.33 ^c	6.2±0.7 ^{bc}	3.8±0.4 ^{bc}
	SBH ₅	334±19 ^d	171±15 ^d	3.49±0.1 ^{cd}	0.71±0.09 ^{cd}	81±6 ^{ab}	33±3 ^{ab}	16±1.0 ^{ab}	10.0±0.38 ^b	6.6±0.4 ^{bc}	4.0±0.2 ^{bc}
	SBH ₁₀	257±14 ^e	150±12 ^e	291±0.1 ^e	0.50±0.06 ^e	88±10 ^a	36±2 ^{ab}	17.2±0.76 ^a	11.7±0.85 ^a	7.5±0.3 ^a	4.5±0.2 ^a

The application of bone-biochar had a significant impact on the yield of plants, as evidenced by the notable enhancement in both root and shoot growth with the use both low and high concentration of bone-biochar, in comparison to the control group (Table 2). The highest percentage increase in shoot length (35%), shoot fresh weight (57%), and shoot dry weight (56%) was observed after the application of the high concentration of bone-biochar, as compared to the control group. Similarly, the root length increased by 65%, root fresh weight by 50% and root dry weight by 45% after the application of SBH-10, while there was a 32% increase in root fresh weight, and 32% increase in root dry weight after the application of SBL-10, in comparison to the control group.

3. Summary and Conclusion

Adding different rates of sheep bone have been enhanced the chemical composition of soil in areas affected by smelter contamination. A pot experiment revealed that the inclusion of SBL at a concentration of 10% led to a reduction in the population of Proteobacteria, which is significant bacterial group. Furthermore, it stimulates soil fertility and augments enzyme levels, thereby facilitating plant growth without any detrimental effects on the environments. The findings of this investigation propose that cow bone-biochar exhibit potential as a fertilizer by promoting enzyme functionality and enhancing plant development.

However, further research is necessary to validate these outcomes under diverse field condition and settings.

4. Future Direction of Work

Due to the tangible ramifications on soil and agricultural produce, it is imperative to establish a linkage between sci-

entific knowledge and practicality by implementing bone char in soil management. In the present era, the proliferation of fertilizer issues has emerged as formidable impediments to crop productivity; thus, it is imperative to prioritize research and development efforts towards soil health.

Consequently, it is recommended to undertake further investigations in order to comprehensively elucidate the impact of diverse sources of bone char on physicochemical attributes, as well as biological and microbial aspects of soil.

Furthermore, exploring the application of bone char, in augmenting crop yield and enhancing its quality warrants through examination.

Abbreviations

CB: Bone Char
 SB: SHEEP Bone
 SBH: Sheep-Derived Bone Biochar Prepared at 800 °C
 SBL: Sheep-Derived Bone Biochar Prepared at 500 °C
 CB: Charred Bone
 BIO: Coffee Husk Biochar
 COM: Compost
 MIN: Mineral Fertilizer
 NS: Non-significance
 K: Potassium
 C: Carbon
 Mg: Magnesium
 Ca: Calcium
 CEC: Cation Exchange Capacity
 N: Nitrogen
 Na: Sodium
 na: Not Determined
 P: Phosphorus
 S: Sulfur
 SOC: Soil Organic Carbon

TP: Total Phosphorus
 DOC: Dissolved Organic Carbon
 TN: Total Nitrogen
 DOM: Dissolved Organic Matter
 EC: Electrical Conductivity
 ND: Not Detected
 SOC: Soil Organic Carbon
 DOC: Dissolved Organic Carbon
 TN: Total Nitrogen
 TP: Total Phosphorus
 UA: Urease
 DA: Dehydrogenase
 BGA: β -glucosidase
 PA: Phosphatase
 SL: Shoot Length
 SFW: Shoot Fresh Weight
 SDW: Shoot Dry Weight
 RL: Root Length
 RFW: Root Fresh Weight
 RDW: Root Dry Weight

Conflicts of Interest

The author declares no conflicts of interest.

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