



Article Combined Effect of Biochar and Fertilizers on Andean Highland Soils before and after Cropping

Tsai Garcia-Perez¹, Manuel Raul Pelaez-Samaniego¹, Jorge Delgado-Noboa¹ and Eduardo J. Chica^{2,*}

- ¹ Department of Applied Chemistry and Systems of Production, Faculty of Chemical Sciences, Universidad de Cuenca, Cuenca 010107, Ecuador; tsai.garcia@ucuenca.edu.ec (T.G.-P.); manual palagz@ucuenca.edu.ec (M.R.P.S.); iarga dalgada@ucuenca.edu.ec (T.D.N.)
 - manuel.pelaez@ucuenca.edu.ec (M.R.P.-S.); jorge.delgado@ucuenca.edu.ec (J.D.-N.)

² Faculty of Agricultural Sciences, Universidad de Cuenca, Cuenca 010107, Ecuador
 * Correspondence: eduardo.chica@ucuenca.edu.ec

Abstract: Although a number of works present biochar as a promising material for improving the quality of degraded soils, only a few show the effect of this material in soils from the Andean highlands. The objective of this work was twofold: (a) to study the effect of two types of biochars on two agricultural soils commonly found in the Andean highlands (Andisol and Inceptisol) and the corresponding soil-biochar-fertilizer interactions, and, (b) to assess the response to biochar of two vegetable crops (lettuce and radish) grown in succession in a simulated double-cropping system. Biochar was produced at 400 °C and 500 °C, for 1 h (B400 and B500, respectively), using hardwood residues. Properties of biochar that could potentially affect its interaction with soil and water (e.g., functional groups, surface area, elemental composition) were assessed. Experiments were conducted to test for main and interaction effects of biochar type, soil type, and the addition of NPK fertilizer on the soils' characteristics. Bulk density and water content at field capacity and permanent wilting point were affected by two-way interactions between biochar and soil type. Biochar impacted bulk density and water retention capacity of soils. Higher available water content was found in soils amended with B400 than with B500, which is a consequence of the higher hydrophilicity of B400 compared to B500. After the lettuce crop was planted and harvested, the soil pH was unaffected by the biochar addition. However, after the second crop, the pH in the Inceptisol slightly decreased, whereas the opposite was detected in the Andisol. The CEC of the Inceptisol decreased (e.g., from 36.62 to 34.04 and from 41.16 to 39.11 in the control and in the Inceptisol amended with B400 only) and the CEC of the Andisol increased (e.g., from 74.25 to 90.41 in the control and from 79.61 to 90.80 in the Andisol amended with B400 only). Inceptisol amended only with biochar showed decrease of radish weight, while a large increase was found in B400 + fertilizer Inceptisol (i.e., from 22.9 g to 40.4 g). In Andisol, the weight of radish after the second crop increased in less proportion (i.e., from 43 g in the control to 59.7 g in the B400 + fertilizer Andisol), showing a visible positive impact of B400. The results suggest that biochar produced at 400 $^{\circ}$ C performs better than biochar produced at 500 °C because B400 apparently promotes a better environment for bacteria growth in the soils, as a consequence of more OH available groups in B400 and its better interaction with water and the fertilizer.

Keywords: Andisol; Inceptisol; wood biochar; soil amendment; water retention capacity; crop yield

1. Introduction

Population growth has been a driving force for the expansion of urban areas worldwide, causing a reduction in available agricultural lands [1–3]. This has affected large parts of arable lands that have been engulfed by growing urban centers in the last three decades. A decrease in available arable soil promotes deforestation for agriculture and cattle pasture use, expansion into erosion-prone (due to rugged topography and steepness) highlands [4–7] and soils with low nutrients content, which is the case of some places in the



Citation: Garcia-Perez, T.; Pelaez-Samaniego, M.R.; Delgado-Noboa, J.; Chica, E.J. Combined Effect of Biochar and Fertilizers on Andean Highland Soils before and after Cropping. *Sustainability* 2022, *14*, 8912. https:// doi.org/10.3390/su14148912

Academic Editor: Salvatore Cataldo

Received: 29 May 2022 Accepted: 18 July 2022 Published: 21 July 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Andean highlands [8]. Soil erosion in the Andean highlands is accompanied by difficulty to retain water. Reforestation could help to reduce erosion, but the thin soil covers in steep lands limits these efforts. Moreover, a method frequently used for augmenting the nutrients content in soil is through the addition of nitrogen-based synthetic fertilizers. However, this method for soil improvement is also contributing to further soil acidification since N-based fertilizers can increase soil acidification [9–11]. Soils in the Andean highlands are commonly characterized by low pH (in some places below 5) [12–14]. Reducing soil erosion and, simultaneously, increasing soil fertility, retaining moisture in the soil, and avoiding soil acidification are challenging tasks in these types of soils.

Biochar has been identified as a promising material for agricultural soil improvement [15-18] and carbon storage in biochar amended soils [19]. For soils such as those found in parts of the Andean highlands, biochar could help to retain moisture and, thus, improve soil fertility. Multiple factors must be considered, however, when assessing soil's response to biochar [16,17,20–22]. Thus, specific studies are necessary to evaluate the properties of the Andean soils affected by biochar and determine the related impacts. The methods used to produce the soil-biochar amendment must also be considered [23]. In Ecuador, a previous work assessed the benefits of using up to 6 t ha⁻¹ of biochar in soils in the Ecuadorian Southern Amazonian region, showing that biochar plus fertilizer contributed to faster growing rates of *Gmelina arborea* trees [24]. The authors used charcoal obtained from a local market, which in Ecuador is produced using rudimentary kilns [25]. Thus, little is known about the characteristics of this biochar. Because different biochar production conditions such as temperature and time lead to biochars with diverse characteristics, different types of biochar-soil interactions can promote specific responses in soil characteristics [16] and plants' responses. Biochar type and dosage, as well as soil type and the corresponding interactions, impact soil fertility and performance [18].

Although previous studies have analyzed the effect of biochar in Andisol and Inceptisol [23,26], only a few have analyzed the effect of biochar in these soils in the conditions of the Andean highlands. Andisols from the Ecuadorian paramos are susceptible to irreversible changes upon drying, which is expected to negatively affect its physical properties [27]. And isols are also characterized by a large porosity [27] and pH below 5 [14,27]. Although it is expected that biochar could help to reduce such susceptibility by improving water retention capacity and, thus, helping to improve the quality of these soils, more work is necessary to better understand the dosages and interactions of biochars with these types of soils and the addition of fertilizers. The strategy for storing C in a stable manner and for long times via biochar addition to soils [19] is of practical interest at the farmers level only if there is an agronomic value of biochar in the soils; i.e., if the fertilizer value of biochar and its positive effects on soil physical properties are confired [26]. Farmers in the Ecuadorian Andes grow a variety of crops, among which vegetables are of great economic and ecological importance due to their market value and the technological intensity required for their production [28,29]. The intensity of use of the soils dedicated to vegetable production (frequent tillage, use of labile forms or organic matter, and frequent irrigation), offers an appropriate setting for developing better soil conservation strategies and selection of amendments with potential soil-transforming effects such as biochars. Therefore, more work is necessary to better understand these benefits, especially in the conditions of soils in the Andean highlands. The objective of this work was twofold: (a) to study the effect of two types of biochars on two agricultural soils commonly found in the Andean highlands (Andisol and Inceptisol) and the corresponding soil-biochar-fertilizer interactions; and, (b) to assess the response to biochar of two vegetable crops grown in succession in a simulated double-cropping system. The work aims to explore the consistency of biochar amendments at producing desired responses in Andean soils' properties and vegetable growth (lettuce and radish) under local conditions.

2. Materials and Methods

2.1. Biochar Production and Characterization

Biochar was produced using wood residues from the wood furniture industry in Cuenca (where ~65% of the wood furniture in Ecuador is manufactured). Wood is one of the most common raw materials used for biochar production intending soil amendment [16]. Approximately 30 kg of wood residues were collected from a small carpentry shop (location $2^{\circ}55'8.8''$ S, $79^{\circ}1'8.1''$ W) and stored at room conditions prior to characterization and carbonization. Wood residues were constituted by a mix of shavings of three commonly used wood species for furniture in Ecuador (Tratinnickia glaziovii, Cedrelinga catenaeformis, and Ocotea javitensis, approximately in equal parts). Prior to pyrolysis, the wood residues were dried (at 103 °C, 24 h) following ASTM D4442-07. Biochar was produced using a custom-made electrically heated oven (internal dimensions $250 \times 250 \times 250$ mm, 3000 W, electronically controlled) (See Figure 1). Two temperatures were selected for pyrolysis: 400 °C and 500 °C, for 1 h at isothermal conditions. The products are herein referred to as B400 and B500, respectively. These temperatures were selected based on previous works on evaluating biochar for soil amendment [30-34]. The heating rate was approximately 12 °C min⁻¹ but the cooling down process was slower. Nitrogen (99.9% purity) (INDURA, Guayaquil-Ecuador) was used as a carrier gas (flow of 4 L min⁻¹). This relatively high N_2 flow intended to reduce tar formation and help tar dragging during the pyrolysis process. Needle valves and a gas flowmeter were employed to regulate the nitrogen flow. Prior to the pyrolysis process, the oven was flushed with nitrogen for 10 min (with the wood shavings previously loaded). The resulting chars were then ground to reduce and homogenize particle size between 20 and 60 mesh. A schematic showing the whole experimental work and related activities is presented in Figure 1.

Proximate analysis (volatiles and fixed carbon content) of B400 and B500 biochars was conducted employing a Mettler Toledo thermogravimetric analyzer (TGA-SDTA851e). Ash content was determined by burning 1 g of material at 600 °C for 2 h (ASTM E1755-01). Elemental analysis (C, H, N content) was measured using a LECO[®] TruSpec CHN analyzer coupled with a LECO[®] 628S detector module [35]. Oxygen was calculated by difference, using the ash content to correct the results of the elemental composition analysis. All tests were conducted in duplicates. Functional groups on the surface of the biochars were detected via Fourier Transform Infrared Spectrometry (FTIR), using a Shimadzu IRAffinity-1 spectrometer operating in the 4000–400 cm⁻¹ range of wave numbers. The apparent surface area of the biochars was studied by gas physisorption analysis using CO₂ as adsorptive gas in a Micrometrics Tristar II Plus equipment (Norcross, GA, USA) at 0 °C (273 K). Prior to the tests, the biochar samples were outgassed under vacuum (5–10 Pa) at 200 °C for 18 h. CO₂ adsorption isotherms were measured between the partial pressure range of $p/p_0 = 10^{-5}$ to $p/p_0 = 0.03$ using 75 set equilibration points. The corresponding surface area and micropore volumes were estimated using the Dubin–Radushkevich theory [36].

2.2. Experimental

A completely randomized factorial experiment was conducted to test for main and interaction effects of biochar type, soil type, and NPK fertilizer addition on physical, chemical, and microbiological characteristics of two types of Andean soils, as well as the response of two vegetable crops grown in succession in a simulated double-cropping system. The types of soils selected were an Inceptisol and an Andisol, which are commonly used for agriculture in the Andean highlands and used for vegetable crops around the city of Cuenca, Ecuador. The soil samples were stored under dry greenhouse conditions (at temperature up to ~25 °C) for approximately two months prior to use. The two types of biochar (B400 and B500) were tested on these soils with and without the NPK fertilizer. The experiment was conducted between May and August 2019 in a greenhouse facility at the Faculty of Agricultural Sciences of the University of Cuenca, using 6 L pots (height 210 mm, upper diameter 220 mm, and base diameter 170 mm). The first crop planted was lettuce (cv. 'Great Lakes' 366, started from transplants) immediately followed by radish

(cv. 'Crimson Giant', started from seeds). In the treatments that received NPK fertilization, the fertilizer was mixed with the soil prior to the first crop (i.e., lettuce). No additional fertilizer was applied after the radish was planted. Irrigation was provided daily by means of micro-sprinklers.



Figure 1. Schematic showing the entire experimental work.

2.3. Treatments of Soil with Biochar

Treatments consisted of all possible combinations of: (a) three levels of biochar amendment (i.e., B400 (biochar produced at 400 °C for 1 h), B500 (biochar produced at 500 °C for 1 h), and no amendment), (b) two types of soil (Inceptisol and Andisol), and (c) two levels of fertilization (with or without NPK fertilizer, applied prior to the first crop), resulting in 12 total treatments. The pots were filled with 2 kg of substrate prepared as indicated by the treatment. Both biochars constituted 5% (100 g) of the substrate weight (dry basis). Fertilizer represented 1% (20 g) of the total weight of the substrate. The fertilizer applied was a granular 15-15-15 NPK. Seven replicate pots per treatment were prepared.

2.4. Soil Physical, Chemical, and Microbiological Properties

Soil physical properties were determined on samples of three randomly selected replicates at the end of the second crop (to avoid major perturbation of the soil structure) using standard methods [37]. Soil bulk density was determined gravimetrically using undisturbed cores. The cores were also used to determine the volumetric soil water content at field capacity (θ_{CC}) and permanent wilting point (θ_{PMP}). Total available water was calculated from the difference between θ_{CC} and θ_{PMP} . Soil chemical properties (pH, organic matter content, N, P, K, Ca, Mg, Zn, Cu, Fe, and Mn) were measured on samples of three replicates at the end of the first and second crop. Soil samples were collected and sent for analysis to an external service provider (Instituto Nacional de Investigaciones Agropecuarias–INIAP, Gualaceo-Ecuador). Soils were extracted using Olsen's solution. N and P were determined colorimetrically using the Kjeldahl and the molybdenum-vanadate method, respectively. Metallic elements were determined by atomic absorption flame spectrometry [38]. Organic matter content was determined by wet oxidation [39]. Culturable bacteria were enumerated in three of the seven replicates using serial dilutions of a soil suspension on phosphate saline buffer plated on nutrient agar [37]. Plates were incubated at room temperature (20 ± 2 °C) for one week before enumeration. Dilutions with 30–200 colonies were used for enumeration. Cation exchange capacity (CEC) of soils was measured following standard procedures (i.e., Ammonium chloride—NH₄Cl method).

2.5. Crop Fresh Weight Measurements

Crop fresh weight was measured on six replicates after the completion of the typical crop cycle for local conditions (8 weeks after transplant for lettuce and 6 weeks after sowing for radish). For lettuce, plants were harvested at the crown and weighted individually, whereas for radish, plants were uprooted, cleaned, and weighted.

2.6. Data Analysis

Data were analyzed using ANOVA after checking for model assumptions. For some variables, outliers were detected and had to be extracted from the dataset to satisfy normality or homoscedasticity assumptions. In the case of the weight of lettuce plants, data were log-transformed to achieve normality because of the large differences in the range of weights between the plants cultivated in the Inceptisol and the Andisol. Statistical analyses were conducted in R [40].

3. Results

3.1. Biochars and Soils Properties

The yields of biochars produced at 400 °C and 500 °C for 1 h, i.e., B400 and B500, were 32.17 ± 1.03 mass% and 30.39 ± 0.51 mass%, in dry basis, respectively. Although the only difference between the biochars' production process was temperature (100 °C higher in B500 than in B400), important differences in the products' properties are seen in Table 1. Temperature is the most important parameter to control the slow pyrolysis process [41] and, thus, the properties of biochar. This explains the differences in the results of both proximate and elemental analyses of biochar (Table 1). In addition, slow pyrolysis and long residence

time result in lower amounts of polycyclic aromatic hydrocarbons (PAHs) in biochars than in fast pyrolysis [42,43].

Table 1. Characteristics of the two biochars used in the work (dry basis).

	B400	B500
Volatiles (% w w^{-1})	31.67 *	27.50
Fixed carbon (% w w ^{-1})	66.97	70.75
Ash (% w w ⁻¹)	1.37	1.75
$C (\% \text{ w } \text{w}^{-1})$	70.49	74.78
$H (\% w w^{-1})$	3.69	3.03
$N (\% w w^{-1})$	0.36	0.58
$S(\% w w^{-1})$	BDL **	BDL
$O(\% w w^{-1})$	25.46	21.61
H/C	0.052	0.041
O/C	0.361	0.289
(O + N)/C	0.366	0.297

* Results are the mean of duplicates. ** Below detection limit.

A reduction of volatiles relative content of the biochars (from ~31.7% to 27.5%) when the pyrolysis conditions changed from 400 °C to 500 °C was observed, suggesting that mass loss during pyrolysis in large part results from volatiles reduction. As expected, the relative content of fixed carbon increased (from ~67% to ~71.8%) when the pyrolysis temperature increased. The results of the elemental composition analysis (Table 1) revealed absence of S in the biochar samples, as expected in most lignocellulosic materials. The mass fraction of H and O decreased from 3.69% to 3.03% and from 25.5% to 21.6%, respectively. The reductions of both H and O result from the cleavage and cracking of weak O bonds within the material, whose products are lost within the pyrolysis gases [32]. The H/C and O/C atomic ratios decrease when the pyrolysis temperature increase. These ratios can be used as indicators of the degree of aromaticity of biochar [44]; thus, B500 presents higher aromaticity than B400. Aromatics in biochar are formed during the pyrolysis process; thus, studies about potential health risks resulting from polycyclic aromatic hydrocarbons (PAHs) in soils amended with biochar have been conducted, showing that the concentration of PHAs in these soils is up to two orders of magnitude below the limits of prevention, according to European and Brazilian legislations [45]. The O/C atomic ratio decreases in B500 compared to B400, indicating that less polar functional groups are present in B500 surface compared to B400. The (O + N)/C ratio also decreased when the pyrolysis temperature was augmented, indicating a higher amount of polar functional groups on the surface of B400, compared to B500.

Both B400 and B500 biochars used in this work are in the range of pyrolysis temperatures that offer the most desirable hydrological properties in biochar [34]. The functional groups on the samples of B400 and B500 biochars and raw material are shown in Figure 2a. The characteristic board peak due to the presence of the O–H bond stretching can be observed at ~3450 cm⁻¹ [46], corresponding to the presence of water on the biochar surface. The peaks at 2972 cm⁻¹ and 1705 cm⁻¹ can be attributed to the presence of the carbonyl bonds (C=O) corresponding to the ketone group and carboxyl group, respectively. The C=O presence on the biochar surface act as a water binding center. The peak at 1600 cm⁻¹ is ascribed to stretching vibrations of the aliphatic –C=C– bond distinctive to biochar [47]. The aliphatic peak at 2800–3000 cm⁻¹ that is identified in the raw material disappears in the spectra of both B400 and B500, suggesting that the hydrophobicity of both biochars is low [34,48]. Although both samples present approximately similar surface chemistry, the peaks intensity decreases at the higher pyrolysis temperature, indicating lower presence of functional groups on the surface of B500.



Figure 2. (a) Functional groups on B400 and B500 biochars' and raw material's surfaces, (b) CO_2 sorption isotherms for the two types of chars.

Figure 2b shows the CO₂ adsorption isotherms for both biochars. The corresponding analysis showed that, for CO₂ adsorption, the surface areas are 274 and 292 m² g⁻¹ for B400 and B500, respectively. Therefore, a small increase of surface area occurs when the pyrolysis temperature changes from 400 to 500 °C. The total pore volumes are 0.06966 cm³ g⁻¹ (at relative pressure 0.029963) and 0.6599 cm³ g⁻¹ (at relative pressure 0.029952) for B400 and B500, respectively. The maximum pore diameter was 0.7967 nm in both cases.

The characteristics of the two soils used in the work are shown in Table 2. The bulk density of Inceptisol was larger than that of Andisol (i.e., $1.04 \text{ g cm}^{-3} \text{ vs.} 0.66 \text{ g cm}^{-3}$). Water content at field capacity (θ_{CC}) and at permanent wilting point (θ_{PMP}), and total available water followed the same trend. Andisol was acidic (pH ~ 5), which is in agreement with results previously reported for these types of soils [14], while Inceptisol's pH was close to 7. The CEC of Andisol (74.25 cmol(+) kg⁻¹) can be rated as very high and that of Inceptisol (36.62 cmol(+) kg⁻¹) as high [49]. The chemical composition shows different nutrients content (higher presence of K, Ca, Mg, and Mn in the Inceptisol than in the Andisol). The bacterial counts were not appreciably different. It is also observed that the organic matter content was higher in the Andisol (32.83%) than in the Inceptisol (7.28%).

	Inceptisol	Andisol
Texture	Loam	Sandy loam
Bulk density (g cm ⁻³)	1.04	0.66
$\theta_{CC} (m^3 m^{-3})^{-3}$	0.49	0.32
$\theta_{PMP} (m^3 m^{-3})$	0.32	0.29
Total available water (m ³ m ⁻³)	0.17	0.03
pH	6.64	5.12
\overline{CEC} (cmol(+) kg ⁻¹)	36.62	74.25
Organic Matter (%)	7.28	32.83
N (ppm)	24.12	34.78
P (ppm)	86.3	10.4
K (ppm)	676.43	191.59
Ca (ppm)	4488.96	1092.18
Mg (ppm)	675.54	140.94
Zn (ppm)	8.1	2.3
Cu (ppm)	2.4	3.3
Fe (ppm)	9.0	110.0
Mn (ppm)	23.2	6.4
Bacterial counts ($\log_{10} \text{UFC} \cdot \text{g}^{-1}$)	5.11	5.43

Table 2. Physical, chemical, and microbiological characteristics of the two soils used in the experiments.

Nomenclature: θ_{CC} —Water content at field capacity; θ_{PMP} —Water content at permanent wilting point.

3.2. *Biochar Effects on Physical, Chemical, and Microbiological Characteristics of Soil* 3.2.1. Soil Physical Properties

Biochar type, soil type, and fertilizer addition interacted to modify the bulk density, θ_{CC} , θ_{PMP} , and total available water after the second crop (Table 3). Bulk density, θ_{CC} , and θ_{PMP} were affected by two-way interactions between biochar and soil type. The bulk densities of the soils amended with both biochars were lower than those of the unamended soils (e.g., the bulk density of Inceptisol amended with B400 and fertilizer was 0.85 g cm^{-3} , whereas the density of the control was 1.06 g cm^{-3}), which results mostly from the low biochar densities. Moreover, the Inceptisol amended with B400 had a greater bulk density than the soil amended with B500 but the opposite was observed with Andisol. Conversely, less clear effects were detected for θ_{CC} and θ_{PMP} . The θ_{CC} was greater in soils amended with either biochar in the Inceptisol, without apparent differences caused by the biochar type. Nevertheless, in the Andisol amended with biochar, the addition of B400 only (i.e., without fertilizer) produced θ_{CC} similar to the controls, whereas B400 with fertilizer addition, and B500 (with or without fertilizer) had less θ_{CC} than the controls. The θ_{PMP} showed a similar and more consistent trend than that of θ_{CC} , with greater θ_{PMP} in the Inceptisol soils amended with either biochar, and lower θ_{PMP} in the Andisol soils than the controls. Total available water ($\theta_{CC} - \theta_{PMP}$) showed three-way interactions between biochar, soil type, and fertilizer addition, which made it difficult to identify clear trends (Table 3). In the Inceptisol, total available water was similar in all the treatments, whereas in Andisol, soils amended with B400 and the fertilized control contained more available water than the other soils. Higher total available water results in part from the slightly higher hydrophilicity of the B400 biochar compared to B500 biochar.

Table 3. Hydrophysical properties of the soils amended with B400 and B500 with (+Fert.) and without fertilizer (–Fert.) and *p*-values for main and interaction effects derived from a biochar \times soil \times fertilizer factorial ANOVA.

			Bulk Density (g cm ⁻³)	θ_{CC} (m ³ m ⁻³)	θ_{PMP} (m ³ m ⁻³)	Total Available Water (m ³ m ⁻³)
	00	+Fert.	0.85 ± 0.02	0.55 ± 0.03	0.45 ± 0.00	0.09 ± 0.00
	B4	-Fert.	0.89 ± 0.05	0.56 ± 0.01	0.44 ± 0.00	0.12 ± 0.00
tisol	00	+Fert.	0.78 ± 0.04	0.56 ± 0.02	0.45 ± 0.01	0.11 ± 0.01
Icep	B5	-Fert.	0.83 ± 0.01	0.58 ± 0.09	0.39 ± 0.06	0.19 ± 0.06
Ч —	ntrol	+Fert.	1.06 ± 0.06	0.47 ± 0.01	0.33 ± 0.03	0.14 ± 0.02
	Coi	-Fert.	1.01 ± 0.03	0.47 ± 0.01	0.36 ± 0.03	0.11 ± 0.03
	00	+Fert.	0.42 ± 0.02	0.58 ± 0.03	0.47 ± 0.05	0.11 ± 0.01
	B4	-Fert.	0.42 ± 0.01	0.73 ± 0.00	0.5 ± 0.01	0.22 ± 0.01
isol	00	+Fert.	0.47 ± 0.01	0.54 ± 0.00	0.42 ± 0.04	0.12 ± 0.04
Andi	B5	-Fert.	0.45 ± 0.00	0.52 ± 0.01	0.46 ± 0.05	0.06 ± 0.05
	ntrol	+Fert.	0.48 ± 0.01	0.73 ± 0.05	0.53 ± 0	0.2 ± 0.00
	Coi	-Fert.	0.54 ± 0.02	0.65 ± 0.05	0.54 ± 0.01	0.11 ± 0.01

		Bulk Density (g cm ⁻³)	θ_{CC} (m ³ m ⁻³)	$ heta_{PMP}$ (m ³ m ⁻³)	Total Available Water (m ³ m ⁻³)
	В	<0.01	0.31	0.35	0.64
	S	<0.01	< 0.01	<0.01	0.82
A/A	F	0.47	0.65	0.61	0.98
0	$B \times S$	< 0.01	< 0.01	< 0.01	0.03
AN	$B \times F$	0.89	0.12	0.77	0.01
	$S \times F$	0.98	0.92	0.31	0.19
	$B \times S \times F$	0.12	0.17	0.50	0.03

Table 3. Cont.

Values are means \pm SE (Standard error) (n = 3). Nomenclature: B—Biochar, S—Soil, F—Fertilizer. Highlighted cells in the Table indicate key results in this set of variables. For interpretation, significant higher order interactions prevail over lower order interactions and main effects.

3.2.2. Soil Chemical Properties

Table 4 shows the CEC results corresponding to the soils after the amendment (second column) and after the second crop (third column). It is seen that, for Inceptisol, in general, CEC decreases after the second crop, regardless of the soil treatment. For example, the CEC decreases from 36.62 to 34.04 in the control, from 41.16 to 39.11 in the Inceptisol amended with B400 only, and from 43.54 to 39.15 in the Inceptisol amended with B500 only. However, in general, Andisol shows an increasing tendency (e.g., CEC increases from 74.25 to 90.41 in the control, from 79.61 to 90.80 in the Andisol amended with B400 only, and from 79.60 to 93.53 in the Andisol amended with B500 only), which is in agreement with pH increases reported in Table 5. CEC is an important factor that helps to control the stability of soil structure, as well as nutrient availability for plant growth, the pH of soils, and the reaction of soils to fertilizers and other materials used as soil amendments [49].

Table 4. Cation exchange capacity of soils.

Type of Soil and/or Preparation	CEC of the Initial Soils *	CEC after the Radish Crop	Tendency
Inceptisol alone	36.62	34.04	Decrease
Inceptisol + fertilizer	38.18	40.62	Increase
Inceptisol + B400	41.16	39.11	Decrease
Inceptisol + B400 + fertilizer	45.53	42.82	Decrease
Inceptisol + B500	43.54	39.15	Decrease
Inceptisol + B500 + fertilizer	49.82	38.04	Decrease
Andisol alone	74.25	90.41	Increase
Andisol + fertilizer	77.77	91.50	Increase
Andisol + B400	79.61	90.8	Increase
Andisol + B400 + fertilizer	79.28	92.85	Increase
Andisol + B500	79.6	93.53	Increase
Andisol + B500 + fertilizer	111.31	93.89	Decrease

* Results are the mean of two measurements.

Several statistically significant interactions between biochar, soil type, and fertilizer were detected among the chemical properties evaluated after the lettuce (Table 5) and the radish crop (Table 6) were harvested. After the lettuce crop was harvested, no changes were noted to the pH due to the addition of biochar, although broader changes were noted, caused by soil type and fertilizer. A significant interaction between biochar and soil type was detected for the soil organic matter content (Table 5). Due to this interaction, soil organic matter was lower in the Inceptisol amended with B500 than in both the B400-amended and control soils, but this trend was not detected in the Andisol. Neither main nor interactive effects of biochar were detected for the residual N, P, K, and Mn content in the soil; nonetheless, significant biochar-soil and biochar-soil–Fertilizer interaction were detected for the Ca, Zn, Mg, and Fe contents of the soils (Table 5).

			pН	OM (%)	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
	00	+Fert.	5.6 ± 0.3	6.7 ± 0.2	17.5 ± 1.3	302.3 ± 10.7	1166.5 ± 394.4	3241.1 ± 246.6	394.9 ± 18.2	8.1 ± 0.4	2.7 ± 0.1	41.7 ± 4.7	3.9 ± 1.5
Ы	B4	-Fert.	6.9 ± 0.1	6.9 ± 0.4	14.2 ± 1.8	98.8 ± 5.9	308.9 ± 13.5	3166.3 ± 98.9	403 ± 4.9	8 ± 0.6	2.5 ± 0.2	25.3 ± 3.9	4.2 ± 0.3
ptise	00	+Fert.	6.1 ± 0.5	6.0 ± 0.2	27 ± 10.9	237.8 ± 73.6	959.3 ± 381.8	4021.4 ± 66.8	435 ± 25.7	9.9 ± 0.2	3 ± 0.2	25 ± 4.2	5.8 ± 2.1
Ince	B5	-Fert.	6.6 ± 0.5	6.0 ± 0.0	18.2 ± 4.4	167 ± 72.5	547.4 ± 207.2	3954.6 ± 26.7	422.4 ± 3.9	10.1 ± 0.5	3.1 ± 0.4	25 ± 5.5	4 ± 0.7
<u> </u>	ntrol	+Fert.	6.1 ± 0.1	7.3 ± 0.4	14.4 ± 0.6	303.7 ± 1.3	803.5 ± 36.7	4068.1 ± 41.7	445.9 ± 27.4	9 ± 0.2	2.5 ± 0.1	25.3 ± 1.2	8.3 ± 1.4
	Coi	-Fert.	7.1 ± 0.1	6.7 ± 0.3	9.9 ± 2.5	73.1 ± 2.8	362.3 ± 19.2	4094.8 ± 247.2	515.6 ± 11.3	8.2 ± 1.1	2.3 ± 0.1	14.3 ± 4.8	4 ± 0.3
	100	+Fert.	4.8 ± 0.3	33.3 ± 0.8	16.1 ± 2.1	35.9 ± 4.2	453.6 ± 82.9	416.8 ± 8.3	100 ± 1.5	2.4 ± 0.5	3.5 ± 0.1	111.3 ± 9.8	3.6 ± 0.4
	B4	-Fert.	5.0 ± 0.1	33.8 ± 0.3	29.3 ± 8.2	10.8 ± 1.4	87.3 ± 10.2	374.7 ± 43.1	73.7 ± 5.7	1.6 ± 0.1	4.2 ± 0.3	132 ± 3	3.4 ± 0.4
disol	lisol 500	+Fert.	4.7 ± 0.0	36.6 ± 0.4	102.1 ± 78.1	38.8 ± 1.3	445.7 ± 27.4	432.2 ± 11.6	104.5 ± 1.2	1.8 ± 0.1	4.4 ± 0.2	151.3 ± 7.2	3 ± 0.2
An	н	-Fert.	5.6 ± 0.2	36.3 ± 0.6	20.3 ± 3.1	10.1 ± 0.9	113.4 ± 11.7	564.5 ± 104.1	98.8 ± 11.5	2.1 ± 0.2	4.3 ± 0.1	141 ± 4.6	3.6 ± 0.6
-	ntrol	+Fert.	4.4 ± 0.1	32.4 ± 0.5	21.5 ± 3.8	39.2 ± 6	434 ± 18.5	378.8 ± 9.3	98 ± 0.8	1.7 ± 0.1	4.3 ± 0.0	149.3 ± 2.4	3.3 ± 0.3
	Õ	-Fert.	4.8 ± 0.1	34.4 ± 0.0	15.1 ± 1.2	8.5 ± 2.8	53.4 ± 1.3	342.7 ± 11.2	71.7 ± 2.4	1.5 ± 0.1	4.0 ± 0.1	114.7 ± 2.7	3.3 ± 0.4
		В	0.55	< 0.01	0.23	0.94	0.50	< 0.01	< 0.01	0.02	< 0.01	0.03	0.36
		S	< 0.01	< 0.01	0.21	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
	-	F	< 0.01	0.20	0.26	< 0.01	< 0.01	0.88	0.88	0.41	0.91	0.01	0.10
	7AC	$B \times S$	0.07	< 0.01	0.53	0.94	0.85	< 0.01	< 0.01	0.02	0.09	< 0.01	0.24
	ĭ	$B \times F$	0.98	0.31	0.28	0.16	0.59	0.85	0.18	0.48	0.14	0.01	0.23
	A	$S \times F$	0.21	0.07	0.47	< 0.01	0.31	0.67	0.01	1.00	0.39	0.86	0.06
		$B \times S \times F$	0.13	0.05	0.36	0.16	0.63	0.71	0.04	0.62	0.06	< 0.01	0.20

Table 5. Properties of the soils amended with B400 and B500 biochars with (+Fert.) and without fertilizer (–Fert.) after the lettuce crop (first crop) and *p*-values for main and interaction effects derived from a biochar × soil × fertilizer factorial ANOVA.

Values are means \pm SE (Standard error) (n = 3). Nomenclature: B—Biochar, S—Soil, F—Fertilizer, OM—Organic matter content. Highlighted cells in the Table indicate key results in this set of variables. For interpretation, significant higher order interactions prevail over lower order interactions and main effects.

			pН	OM (%)	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
	00	+Fert.	6.2 ± 0.2	6.3 ± 0.1	108.3 ± 81.9	9 283.7 ± 12.7	981.4 ± 224.8	4254.5 ± 130.9	392 ± 22.1	17.8 ± 0.9	4.7 ± 0.1	131 ± 19.2	11.3 ± 3
	B4	-Fert.	7.2 ± 0.0	7.0 ± 0.1	75.6 ± 26.9	168.2 ± 40.9	521.3 ± 76.1	3989.3 ± 191.4	477.5 ± 25.8	26.5 ± 0.4	4.3 ± 0.8	72.3 ± 23.2	7 ± 1.2
sol	00	+Fert.	6.0 ± 0.2	5.9 ± 0.2	76.6 ± 46	312.3 ± 1.3	1050.5 ± 144.6	4419.5 ± 86.4	387.2 ± 10	20.2 ± 1.9	5.5 ± 0.2	147.7 ± 10.9	16 ± 2.5
Incepti	B5	-Fert.	7.2 ± 0.0	6.8 ± 0.3	$\begin{array}{c} 39.3 \pm \\ 15.4 \end{array}$	238.7 ± 2.7	424.9 ± 31.3	3881.1 ± 84	454 ± 13.1	25.7 ± 0.9	5.4 ± 0.1	112 ± 3.6	11.7 ± 0.9
	ntrol	+Fert.	6.4 ± 0.1	6.3 ± 0.0	18 ± 2.2	244 ± 35.9	1063.5 ± 312.3	4236.5 ± 118.4	462.5 ± 10.2	11.9 ± 0.2	2.9 ± 0.0	38.7 ± 3.9	6.7 ± 1.2
Cor	Coi	-Fert.	7.3 ± 0.0	6.2 ± 0.2	80.1 ± 39.2	220.3 ± 13.7	366.2 ± 3.4	3798.9 ± 25.6	530.1 ± 7.8	30 ± 0.6	6.4 ± 0.6	136.3 ± 26.6	12 ± 2.1
	00	+Fert.	4.6 ± 0.3	29.2 ± 0.1	27.1 ± 2.7	64.3 ± 26.4	581.3 ± 67	813 ± 168.8	123.5 ± 25.1	2.7 ± 0.1	7 ± 0.1	361 ± 1.5	4 ± 0.6
1	B4	-Fert.	5.4 ± 0.1	29.9 ± 0.1	$\begin{array}{c} 50.2 \pm \\ 30.9 \end{array}$	3.8 ± 0.3	138.2 ± 13	661.3 ± 84.8	96.8±9	1.8 ± 0.1	5.1 ± 0.4	234.7 ± 11.3	3 ± 0.6
diso	00	+Fert.	5.2 ± 0.1	28.6 ± 0.4	32.9 ± 1.5	50.6 ± 2.8	391 ± 31.4	682.7 ± 45.5	105.7 ± 4.4	2.5 ± 0.2	8.5 ± 0.2	426.7 ± 4.3	3.7 ± 0.3
Ano	B5	-Fert.	5.6 ± 0.1	28.6 ± 0.4	29.2 ± 1.5	11.8 ± 1.8	143.4 ± 16	750.2 ± 69.2	101.2 ± 8.4	2.8 ± 0.2	8.9 ± 0.3	418.7 ± 4.7	3.0 ± 0.0
_	ntrol	+Fert.	5.2 ± 0.1	29.9 ± 0.1	25.2 ± 8.4	29.7 ± 2.7	271.1 ± 65.5	531.1 ± 18	103.3 ± 6.1	2.0 ± 0.0	5.3 ± 0.2	250.3 ± 1.2	3.3 ± 0.3
	Col	-Fert.	5.3 ± 0.0	29.9 ± 0.2	28.5 ± 2.6	7.8 ± 1	73 ± 9.1	557.1 ± 4.2	82.2 ± 2.3	2.3 ± 0.1	8.9 ± 0.2	392.3 ± 0.9	2.3 ± 0.3
		В	0.08	< 0.01	0.46	0.09	0.46	0.05	0.01	< 0.01	< 0.01	< 0.01	0.04
		S	< 0.01	< 0.01	0.08	<0.01	<0.01	<0.01	<0.01	< 0.01	< 0.01	< 0.01	< 0.01
	A	F	< 0.01	0.01	0.89	<0.01	< 0.01	<0.01	<0.01	< 0.01	< 0.01	0.80	0.23
(2 Z	$B \times S$	0.02	< 0.01	0.76	0.15	0.70	0.82	<0.01	0.19	< 0.01	< 0.01	0.05
	AI	$B \times F$	0.06	0.08	0.50	0.06	1.00	0.97	0.92	<0.01	< 0.01	< 0.01	0.04
		$S \times F$	< 0.01	0.26	0.79	0.16	0.05	< 0.01	<0.01	<0.01	0.50	0.92	0.89
		$B \times S \times F$	0.29	0.19	0.42	0.59	0.38	0.25	0.60	< 0.01	0.15	0.01	0.03

Table 6. Properties of the soils amended with B400 and B500 biochars with (+Fert.) and without fertilizer (–Fert.) after the radish crop (second crop) and *p*-values for main and interaction effects derived from a biochar × soil × fertilizer factorial ANOVA.

Values are means \pm SE (Standard error) (n = 3). For Nomenclature see Table 4. Highlighted cells in the Table indicate key results in this set of variables. For interpretation, significant higher order interactions prevail over lower order interactions and main effects.

After the radish crop was harvested (Table 6), biochar–soil interactions were detected in the Inceptisol amended with both types of biochar. Both Inceptisol–biochar mixes had lower pH than the controls, whereas the opposite was observed in the Andisol amended with biochar without fertilizer. Compared to the control, a greater soil organic matter content was detected in the biochar-amended Inceptisol, while less soil organic matter content was detected in the biochar-amended Andisol (both without fertilizer). The addition of fertilizer to biochar amended soils reduced soil organic matter when compared to the unfertilized biochar-amended soils. No biochar-related effects were detected for either the residual N, P, K, and Ca content, whereas biochar–soil and biochar–fertilizer interactions were detected for the residual Mg, Cu, Fe, and Mn content and three-way interactions were detected for the residual soil Zn content (Table 6).

3.2.3. Soil Bacterial Counts

The bacterial count of the two types of soils, treated with biochar and fertilizer addition, and treated with biochar only, are present in Table 7. Although no biochar-related effects were detected on the bacterial counts of either soil after the lettuce crop was harvested, a significant biochar–soil interaction was detected after the radish crop was harvested. For example, the bacterial counts in Inceptisol amended with B400 only, after the radish crop, was 6.6 log₁₀ UFC g⁻¹, while that of the control was 5.4 log₁₀ UFC g⁻¹. The biochar–soil interaction after the second harvest can be attributed to the longer time of biochar in the soil as compared to the first harvest. Bacterial counts in the B500-amended soils after the second harvest were similar to those of the control soil. Nevertheless, B400-amended soils (with and without fertilizer) had markedly greater bacterial counts than the control in the Inceptisol samples, and equal (B400 + fertilizer) or slightly lower (B400) counts than the control in the Andisol samples. It is possible that the B400 biochar promotes a better environment for bacteria in the soils, which could be related to the presence of more OH groups on this biochar's surface.

Table 7. Culturable bacteria counts in the soils amended with B400 and B500 biochars with (+Fert.) and without fertilizer (–Fert.) after two successive crops (lettuce and radish), and *p*-values for main and interaction effects derived from a biochar \times soil \times fertilizer factorial ANOVA.

			Bacterial Counts after First Crop (Lettuce, log_{10} UFC g ⁻¹)	Bacterial Counts after Second Crop (Radish, log ₁₀ UFC g ⁻¹)
	00	+Fert.	5.6 ± 0.2	7.0 ± 0.2
	B4	-Fert.	5.1 ± 0.2	6.6 ± 0.4
tisol	00	+Fert.	5.6 ± 0.3	5.1 ± 0.2
deou	B5	-Fert.	5.2 ± 0.3	5.1 ± 0.2
II —	ntrol	+Fert.	5.1 ± 0.2	5.5 ± 0.4
	Coi	-Fert.	5.2 ± 0.2	5.4 ± 0.4
	00	+Fert.	5.4 ± 0.2	5.6 ± 0.7
	B4	-Fert.	4.7 ± 0.1	4.3 ± 0.4
isol	00	+Fert.	4.6 ± 0.2	5.6 ± 0.2
And	B5	-Fert.	4.9 ± 0.5	5.6 ± 0.1
4	ntrol	+Fert.	5.2 ± 0.0	5.6 ± 0.7
	Coi	-Fert.	4.4 ± 0.2	5.0 ± 0.5

		Bacterial Counts after First Crop (Lettuce, log_{10} UFC g ⁻¹)	Bacterial Counts after Second Crop (Radish, \log_{10} UFC g ⁻¹)
	В	0.44	0.12
	S	<0.01	0.04
AVC	F	0.07	0.07
	$B \times S$	0.54	<0.01
ANG	$B \times F$	0.28	0.33
7	$S \times F$	0.70	0.27
	$B\times S\times F$	0.14	0.72

Table 7. Cont.

Values are means \pm SE (n = 3). Highlighted cells in the Table indicate key results in this set of variables. For interpretation, significant higher order interactions prevail over lower order interactions and main effects.

3.3. Biochar Effects on Plant Growth

The effect of adding biochar to the two types of soils and the resulting interactions are presented in Table 8. Biochar, soil type, and fertilizer produced significant main effects on the growth of lettuce plants. Fertilizer addition resulted in the largest effect on fresh weight of the lettuce plants. In general, biochar-amended soils produced heavier lettuce plants than the control in the Inceptisol without fertilization and in the Andisol (with or without fertilization) (e.g., 65.9 g vs. 46.4 g in B-400 only amended Inceptisol and in the control, respectively). However, the plants grown in B400-amended soils were heavier than those grown in B500-amended soils. After the radish crop harvest, interaction effects between biochar and fertilizer were detected. In part due to this interaction, biochar-amended soils with fertilizer (both Inceptisol and Andisol) produced plants heavier than the controls. As shown in Table 8, the radish weights were 40.4 g and 22.9 g in B-400 + fertilizer Inceptisol and 43.0 g in the B400 + fertilizer and in the control, respectively. Furthermore, the biochar-amended soils without fertilizer produced plants either lighter than (Inceptisol) or similar to (Andisol) the control.

Table 8. Weight of the two crops (lettuce and radish) grown in succession in two soils amended with B400 and B500 biochars with (+Fert.) and without fertilizer (–Fert.), and *p*-values for main and interaction effects derived from a biochar \times soil \times fertilizer factorial ANOVA.

			Lettuce Weight (g)	Radish Weight (g)
	00	+Fert.	194.7 ± 19.7	40.4 ± 10
	B4	-Fert.	65.9 ± 10.7	13.5 ± 6.4
tisol	00	+Fert.	206.8 ± 7	25.3 ± 7.7
Jcep	B5	-Fert.	46.6 ± 6.2	13.4 ± 3.9
Ir	ntrol	+Fert.	200.3 ± 21.1	22.9 ± 5.5
	Coi	-Fert.	46.4 ± 13.2	20.7 ± 6.1
	00	+Fert.	16.0 ± 2.4	59.7 ± 7.5
	B4	-Fert.	3.4 ± 0.6	11.4 ± 4.9
isol	00	+Fert.	13.9 ± 1.4	50.5 ± 4.7
And	B5	-Fert.	3.6 ± 0.4	17.9 ± 7.3
7	ntrol	+Fert.	10.8 ± 2.4	43.0 ± 8.4
	Coi	-Fert.	2.8 ± 0.4	6.0 ± 2.7

Fable 8	B. Cont.
---------	-----------------

		Lettuce Weight (g)	Radish Weight (g)
ANOVA	В	<0.01	0.07
	S	< 0.01	<0.01
	F	<0.01	<0.01
	$B \times S$	0.29	0.33
	$B \times F$	0.92	0.01
	$S \times F$	0.89	<0.01
	$B \times S \times F$	0.08	0.46

Values are means \pm SE (n = 3). Highlighted cells in the Table indicate key results in this set of variables. For interpretation, significant higher order interactions prevail over lower order interactions and main effects.

4. Discussion

There exists a growing global interest on using biochar as a soil amendment due to its capacity to improve soil properties and increase crops production [50,51]. Still, information on biochar-amended soils also reports agriculturally unfavorable effects of biochar, in both crop development and soil properties [52,53]. The differing responses of soils and crops to biochar application suggest that complex interactions between the type of biochar, percent of biochar in the soil, soil properties, climate factors, and plant type exist. In this work, some of these interactions were tested by simulating a rotational cropping system using two soils commonly found in the agriculture of the Andean highlands. Biochar production conditions that could further complicate the responses of soil and crops to the amendment were also tested.

Although the sole difference between the production conditions of the two biochar types was temperature, different responses were produced by the biochar type on soil properties and growth of high rotation plants. The differences between B400 and B500 biochar types include elemental composition (particularly C and O content), degree of aromaticity, hydrophobicity, and surface area. These differences promote visible changes on the responses of the soils to the biochars. Higher hydrophilicity allows better compatibility between water and biochar. Water retention capacity is undoubtedly a factor that positively impacts soil properties and further plants' growth. However, our result suggests that this trend should not always be expected since the type of soil also affects the results. It appears that the main interactions between the biochars (both B400 and B500) and the soil are second order, affecting the bulk density, θ_{CC} , θ_{PMP} , and total available water in the soil. As a consequence of these interactions, the biochar B500 produced more desirable changes (lower bulk density and higher total available water) in the Inceptisol whereas in the Andisol, biochar B400 produced more desirable changes. Although the response to the biochar amendment in the Inceptisol could be labeled as neutral or beneficial to hydrophysical properties, in the Andisol the effects of biochar amendment with B500 could be considered detrimental as it reduced the total available water in the soil, which could in turn affect plant growth. A possible explanation for the better performance of B400 compared to B500 is that B400 is less hydrophobic than B500.

Although biochar can increase total available water in different types of soils [54], lack of effects on total available water have also been reported [30,55]. In our work, increases, no changes, or reductions in total available water depended on the combination of biochar amendment and soil type. Different effects of biochar on the physical properties of different soil types have been reported previously, suggesting that beneficial biochar effects on soil hydrophysical properties would be less pronounced in fine textured soils [51]. Nonetheless, it has also been reported that the hydrophysical properties of silt rich soils could solely be minutely affected by biochar [56]. In our experiment, the Inceptisol and Andisol were loam and sandy loam soils, respectively, and, thus, expected to respond rather well in terms of hydrophysical properties to the application of biochar. The mixed results in this experiment, however, make it difficult to provide general recommendations on the use of biochar to improve the hydrophysical characteristics of the tested soils.

Similar to the response of hydrophysical properties of the soils to the addition of biochar, chemical properties also showed a varied response with significant interactions between the type of biochar, soil, and whether fertilizer was added or not to the pots. In general, biochar is considered to have a liming effect on the soil, increasing its pH [53,57]. This effect has been reported to be dependent on the buffering capacity of the soil and the influence of added fertilizer [50,58,59]. No significant effects (neither main nor interaction effects) of biochar addition were detected on the pH of either soils after the first crop cycle, suggesting that the liming capacity of the tested biochars was limited, even accounting for the rate at which the biochar was applied, which was higher than in other studies where such an effect has been reported [57,59]. However, a significant interaction between the biochar and soil type produced higher pH in the biochar-amended Andisol after the second crop. This result indicates a delayed liming effect that increased the buffering capacity of the soil, which is in agreement with previous observations showing that the soil response to biochar increases over time after the initial application [51]. As expected, the addition of fertilizer reduced the pH of both soils, but this reduction was less marked in the Andisol than in the Inceptisol. Van Zwieten et al. (2010) reported similar results for an acidic Ferrosol and an alkaline Calcarosol in the biochar they produced and attributed this liming effect to Ca complexes formed from $CaCO_3$ present in their feedstock [59]. The feedstock used in their study was different than the one used herein. Nevertheless, in general, biochars contain small amounts of ashes that can contribute bases to neutralize acidic soils [53], which is also our case, since ash content in our biochars can be up to 1.75 mass% (Table 1). Ashes in biochar are also attributed to positively impact CEC of soils.

Another property that showed a marked and contrasting response to the application of biochar in both soils was the organic matter content. Soils that received biochar had less organic matter after the first crop in the Inceptisol, whereas the opposite was observed in the Andisol. Interestingly, after the second crop, this result was reversed and the organic matter content was higher than controls in the biochar amended Inceptisol, while the opposite was identified in the biochar amended Andisol. Still, even though a statistically significant interaction between soil type and biochar addition supports this observation, the magnitude of the change in organic matter in any of the cases is negligible for any practical implication. In general, higher available water content was found in soils amended with B400 than with B500, which is a consequence of the higher hydrophilicity of B400 compared to B500. The results suggest that biochar produced at 400 °C performs better than biochar produced at 500 °C because B400, apparently, promotes a better environment for bacteria growth in the soils, in part as a consequence of more OH available groups in B400 and its better interaction with water and fertilizer.

Generally recognized as stable under soil conditions, a net increase and relatively stable organic matter content was expected in the biochar amended soils. Nevertheless, the addition of biochar could promote degradation of labile carbon [60,61], which could explain the decrease of soil organic matter in our results. Still, the reversal of the original effect in both soils highlights the importance of planning long term experiments and rotations to avoid confusion of transient changes with long term effects of biochar amendments in different types of soils. Biochar amendment effects on bacterial counts were not detected until the end of the second crop when the effect of biochar was strongly dependent on the type of soil, with B400 increasing counts in the Inceptisol and B500 producing no effect, whereas in the Andisol, the opposite was true. The effects of biochar on microbial abundance are mixed and suggest the presence of important interactions between the amendment, the soil type, and the group of microorganisms, since some groups of organisms seem to benefit from the introduction of biochar whereas others do not [62]. This finding is in agreement with results reported in previous works [21].

Biochar has been proposed as a way to increase the CEC of soils and, consequently, increase their capacity to hold nutrients [53]. The consistent increase in Ca, Mg, Zn, Cu, Fe, and Mn concentrations in the B500-amended Andisol could be a consequence of increased cation exchange sites due to biochar addition. However, the lack of consistent response

in the Inceptisol and the B400-amended Andisol are indicative of important soil-biochar interaction effects and the influence of biochar processing conditions in this interaction.

In terms of plant growth, our results show a slight increase in the weight of the crops used in the simulated rotation, with larger effects of fertilizer and soil type on the development of the plants. Such a small effect, however, is in agreement with the estimated effect of biochar applications in other crops. Jeffery et al. estimated in their meta-analysis an average increase in crop productivity by ~10% with a range from -28% to 39% change relative to the controls [52]. In our case, the range of responses was varied, ranging from ~34% reduction in weight of radish in the biochar amended Inceptisol to ~100% increase of weight in radish in the B400 amended Inceptisol with addition of fertilizer (all values relative to the corresponding control).

5. Conclusions

Biochars produced from hardwood residues at 400 °C and 500 °C for 1 h (B400 and B500, respectively) were tested to identify the main and interaction effects of biochar type, soil type, and NPK fertilizer addition on the physical, chemical, and microbiological characteristics of the soils. Results show that biochar alone is not sufficient to promote visible benefits, although it allows better interaction of soils with fertilizers and water. Biochar produced at lower temperatures (i.e., B400) works better than biochar produced at higher temperatures (i.e., B500). The presence of oxygen functional groups and, thus, biochar's hydrophilicity, appears to positively impact biochar properties required for soil amendment. The effect of biochar affinity to soils and water seems to play an important role for this biochar's behavior. However, it appears that some positive effects are not possible to detect in the short term (i.e., in the order of a few weeks, as tested herein). It has also been found that the type of soil responds differently to different types of biochar additions. In the case of degraded soils such as those in some parts of the Andean highlands, the addition of biochar could help to increase the water retention capacity of the soils, a critical factor to support agriculture in steep soils in these regions. Although this study shows some positive effects of B400 and B500 biochar in Andisol and Inceptisol in the Andean highlands, further long-term research is needed for more data acquisition on biochar-soil interactions. Additional benefits of using biochar in soils (e.g., carbon sequestration and soil physical characteristics) need to be tested, preferably under local conditions, in long-term tests.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14148912/s1.

Author Contributions: Conceptualization, T.G.-P., M.R.P.-S. and E.J.C.; methodology, T.G.-P. and E.J.C.; investigation, T.G.-P., E.J.C. and J.D.-N.; resources, T.G.-P.; data curation, E.J.C., T.G.-P. and J.D.-N.; writing—original draft preparation, T.G.-P. and E.J.C.; writing—review and editing, M.R.P.-S. and T.G.-P.; project administration, T.G.-P.; funding acquisition, T.G.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Department of Research of the Universidad de Cuenca (DIUC), through project DIUC-XV-2017-008.

Data Availability Statement: The data presented in this study are available as Supplementary Materials.

Acknowledgments: Thanks to Raul Pelaez-Garcia for English editing and Gabriela Auqui for conducting the carbonization process.

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

References

- 1. He, C.; Liu, Z.; Xu, M.; Ma, Q.; Dou, Y. Urban expansion brought stress to food security in China: Evidence from decreased cropland net primary productivity. *Sci. Total Environ.* **2017**, *576*, 660–670. [CrossRef] [PubMed]
- Ustaoglu, E.; Williams, B. Determinants of Urban Expansion and Agricultural Land Conversion in 25 EU Countries. *Environ. Manag.* 2017, 60, 717–746. [CrossRef] [PubMed]
- 3. Abass, K.; Adanu, S.K.; Agyemang, S. Peri-urbanisation and loss of arable land in Kumasi Metropolis in three decades: Evidence from remote sensing image analysis. *Land Use Policy* **2018**, *72*, 470–479. [CrossRef]
- 4. Ochoa, P.A.; Fries, A.; Mejía, D.; Burneo, J.I.; Ruíz-Sinoga, J.D.; Cerdà, A. Effects of climate, land cover and topography on soil erosion risk in a semiarid basin of the Andes. *Catena* **2016**, *140*, 31–42. [CrossRef]
- 5. Tenorio, G.E.; Vanacker, V.; Campforts, B.; Álvarez, L.; Zhiminaicela, S.; Vercruysse, K.; Molina, A.; Govers, G. Tracking spatial variation in river load from Andean highlands to inter-Andean valleys. *Geomorphology* **2018**, *308*, 175–189. [CrossRef]
- 6. Suquilanda, M. El deterioro de los suelos en el Ecuador y la producción agrícola. In Proceedings of the XI Congreso Ecuatoriano de la Ciencia del Suelo, Quito, Ecuador, 29–31 October 2008.
- 7. Rosas, M.A.; Gutierrez, R.R. Assessing soil erosion risk at national scale in developing countries: The technical challenges, a proposed methodology, and a case history. *Sci. Total Environ.* **2020**, *703*, 135474. [CrossRef]
- 8. Urgilez-Clavijo, A.; Riva, J.; Rivas-Tabares, D.A.; Tarquis, A.M. Linking deforestation patterns to soil types: A multifractal approach. *Eur. J. Soil Sci.* 2021, 72, 635–655. [CrossRef]
- 9. Tian, D.; Niu, S. A global analysis of soil acidification caused by nitrogen addition. Environ. Res. Lett. 2015, 10, 024019. [CrossRef]
- 10. Goulding, K.W.T. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use Manag.* **2016**, *32*, 390–399. [CrossRef]
- 11. Zhang, X.; Guo, J.; Vogt, R.D.; Mulder, J.; Wang, Y.; Qian, C.; Wang, J.; Zhang, X. Soil acidification as an additional driver to organic carbon accumulation in major Chinese croplands. *Geoderma* **2020**, *366*, 114234. [CrossRef]
- 12. Espinosa, J. Distribución, uso y manejo de los suelos de la Región Andina. In Proceedings of the XI Congreso Ecuatoriano de la Ciencia del Suelo, Quito, Ecuador, 29–31 October 2008.
- 13. Cruzatty, L.C.G.; Vollmann, J.E.S. Caracterización de suelos a lo largo de un gradiente altitudinal en Ecuador. *Rev. Bras. De Ciências Agrárias-Braz. J. Agric. Sci.* 2012, 7, 456–464. [CrossRef]
- 14. Quichimbo, P.; Tenorio, G.; Borja, P.; Cardenas, I.; Crespo, P.; Celleri, R. Efectos sobre las propiedades físicas y químicas de los suelos por el cambio de la cobertura vegetal y uso del suelo: Páramo de Quimsacocha al sur del Ecuador. *Suelos Ecuat.* **2012**, *42*, 138–153.
- 15. Lehmann, J.; Stephen, J. Biochar for Environmental Management: Science, Technology and Implementation; Routledge: London, UK, 2015; ISBN 9781844076581.
- Razzaghi, F.; Obour, P.B.; Arthur, E. Does biochar improve soil water retention? A systematic review and meta-analysis. *Geoderma* 2020, *361*, 114055. [CrossRef]
- 17. Kamali, M.; Jahaninafard, D.; Mostafaie, A.; Davarazar, M.; Gomes, A.P.D.; Tarelho, L.A.C.; Dewil, R.; Aminabhavi, T.M. Scientometric analysis and scientific trends on biochar application as soil amendment. *Chem. Eng. J.* **2020**, *395*, 125128. [CrossRef]
- Chen, L.; Liu, M.; Ali, A.; Zhou, Q.; Zhan, S.; Chen, Y.; Pan, X.; Zeng, Y. Effects of Biochar on Paddy Soil Fertility Under Different Water Management Modes. J. Soil Sci. Plant Nutr. 2020, 20, 1810–1818. [CrossRef]
- 19. Lorenz, K.; Lal, R. Biochar application to soil for climate change mitigation by soil organic carbon sequestration. *J. Plant Nutr. Soil Sci.* 2014, 177, 651–670. [CrossRef]
- 20. Alling, V.; Hale, S.E.; Martinsen, V.; Mulder, J.; Smebye, A.; Breedveld, G.D.; Cornelissen, G. The role of biochar in retaining nutrients in amended tropical soils. *J. Plant Nutr. Soil Sci.* 2014, 177, 671–680. [CrossRef]
- Imparato, V.; Hansen, V.; Santos, S.S.; Nielsen, T.K.; Giagnoni, L.; Hauggaard-Nielsen, H.; Johansen, A.; Renella, G.; Winding, A. Gasification biochar has limited effects on functional and structural diversity of soil microbial communities in a temperate agroecosystem. *Soil Biol. Biochem.* 2016, 99, 128–136. [CrossRef]
- 22. Palviainen, M.; Berninger, F.; Bruckman, V.J.; Köster, K.; de Assumpção, C.R.M.; Aaltonen, H.; Makita, N.; Mishra, A.; Kulmala, L.; Adamczyk, B.; et al. Effects of biochar on carbon and nitrogen fluxes in boreal forest soil. *Plant Soil* **2018**, 425, 71–85. [CrossRef]
- 23. Curaqueo, G.; Meier, S.; Khan, N.; Cea, M.; Navia, R. Use of biochar on two volcanic soils: Effects on soil properties and barley yield. *J. Soil Sci. Plant Nutr.* 2014, 14, 911–924. [CrossRef]
- Valarezo, C.A.; Villamagua, M.A.; Mora, P.M.; Maza, H.; Wilcke, W.; Nieto, C. Respuesta del pachaco (Schizolobium parahybum Vell. Conc) y la melina (Gmelina arbórea Roxb.) a la aplicación de biocarbón y fertilización en el sur de la amazonia ecuatoriana. Bosques Latid. Cero 2016, 6, 1.
- 25. Pelaez Samaniego, M.R.; Espinoza Abad, J.L. *Energías Renovables en el Ecuador: Situación Actual, Tendencias y Perspectivas;* Universidad de Cuenca, Graficas Hernandez: Cuenca, Spain, 2015.
- Herath, H.M.S.K.; Camps-Arbestain, M.; Hedley, M. Effect of biochar on soil physical properties in two contrasting soils: An Alfisol and an Andisol. *Geoderma* 2013, 209, 188–197. [CrossRef]
- 27. Poulenard, J.; Podwojewski, P.; Herbillon, A.J. Characteristics of non-allophanic Andisols with hydric properties from the Ecuadorian páramos. *Geoderma* **2003**, *117*, 267–281. [CrossRef]
- 28. Baquero, F.S.; Fazzone, M.R.; Falconi, C. *Politicas Para la Agricultura Familiar en America Latina y el Caribe*; Oficina Regional de la FAO para América Latina y el Caribe: Santiago, Chile, 2007.

- Zea, P.; Chilpe, J.; Sánchez, D.; Eduardo, J. Chica Energy efficiency of smallholder commercial vegetable farms in Cuenca (Ecuador). Tropical and Subtropical Agroecosystems. FAO and IADB. 2020. Available online: https://publications.iadb.org/publications/ spanish/document/Pol%C3%ADticas-para-la-agricultura-familiar-en-Am%C3%A9rica-Latina-y-el-Caribe.pdf (accessed on 28 May 2022).
- Burrell, L.D.; Zehetner, F.; Rampazzo, N.; Wimmer, B.; Soja, G. Long-term effects of biochar on soil physical properties. *Geoderma* 2016, 282, 96–102. [CrossRef]
- 31. Berihun, T.; Tadele, M.; Kebede, F. The application of biochar on soil acidity and other physico-chemical properties of soils in southern Ethiopia. *J. Plant Nutr. Soil Sci.* 2017, *180*, 381–388. [CrossRef]
- 32. Suliman, W.; Harsh, J.B.; Abu-Lail, N.I.; Fortuna, A.M.; Dallmeyer, I.; Garcia-Perez, M. Influence of feedstock source and pyrolysis temperature on biochar bulk and surface properties. *Biomass Bioenergy* **2016**, *84*, 37–48. [CrossRef]
- 33. Mor, S.; Negi, P.; Ravindra, K. Potential of agro-waste sugarcane bagasse ash for the removal of ammoniacal nitrogen from landfill leachate. *Environ. Sci. Pollut. Res.* 2019, *26*, 24516–24531. [CrossRef]
- Kinney, T.J.; Masiello, C.A.; Dugan, B.; Hockaday, W.C.; Dean, M.R.; Zygourakis, K.; Barnes, R.T. Hydrologic properties of biochars produced at different temperatures. *Biomass Bioenergy* 2012, 41, 34–43. [CrossRef]
- 35. Pelaez-Samaniego, M.R.; Perez, J.F.; Ayiania, M.; Garcia-Perez, T. Chars from wood gasification for removing H2S from biogas. *Biomass Bioenergy* **2020**, *142*, 105754. [CrossRef]
- 36. Mood, S.H.; Ayiania, M.; Jefferson-Milan, Y.; Garcia-Perez, M. Nitrogen doped char from anaerobically digested fiber for phosphate removal in aqueous solutions. *Chemosphere* **2020**, 240, 124889. [CrossRef]
- 37. Margesin, R.; Schinner, F. *Manual for Soil Analysis-Monitoring and Assessing Soil Bioremediation*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2005; Volume 5.
- Isaac, R.A. Atomic Absorption Methods for Analysis of Soil Extracts and Plant Tissue Digests. J. AOAC Int. 1980, 63, 788–796.
 [CrossRef]
- 39. Mylavarapu, R.; Sikora, F.J.; Moore, K.P. Soil test methods from the Southeastern United States. *Walkley-Black Method* **2014**, 419, 54–58.
- 40. R Core Team. The R Project for Statistical Computing. Available online: https://www.r-project.org/ (accessed on 14 February 2021).
- 41. Vieira, F.R.; Romero Luna, C.M.; Arce, G.L.A.F.; Ávila, I. Optimization of slow pyrolysis process parameters using a fixed bed reactor for biochar yield from rice husk. *Biomass Bioenergy* **2020**, *132*, 105412. [CrossRef]
- 42. You, S.; Ok, Y.S.; Chen, S.S.; Tsang, D.C.W.; Kwon, E.E.; Lee, J.; Wang, C.H. A critical review on sustainable biochar system through gasification: Energy and environmental applications. *Bioresour. Technol.* **2017**, 246, 242–253. [CrossRef]
- 43. Wang, C.; Wang, Y.; Herath, H.M.S.K. Polycyclic aromatic hydrocarbons (PAHs) in biochar–Their formation, occurrence and analysis: A review. *Org. Geochem.* 2017, 114, 1–11. [CrossRef]
- 44. Wang, Z.; Cao, J.; Wang, J. Pyrolytic characteristics of pine wood in a slowly heating and gas sweeping fixed-bed reactor. *J. Anal. Appl. Pyrolysis* **2009**, *84*, 179–184. [CrossRef]
- 45. de Resende, M.F.; Brasil, T.F.; Madari, B.E.; Pereira Netto, A.D.; Novotny, E.H. Polycyclic aromatic hydrocarbons in biochar amended soils: Long-term experiments in Brazilian tropical areas. *Chemosphere* **2018**, 200, 641–648. [CrossRef]
- 46. Wang, Y.; Xiao, X.; Chen, B. Biochar Impacts on Soil Silicon Dissolution Kinetics and their Interaction Mechanisms. *Sci. Rep.* **2018**, *8*, 8040. [CrossRef]
- 47. Zhang, C.; Zhang, N.; Xiao, Z.; Li, Z.; Zhang, D. Characterization of biochars derived from different materials and their effects on microbial dechlorination of pentachlorophenol in a consortium. *RSC Adv.* **2019**, *9*, 917–923. [CrossRef]
- Gray, M.; Johnson, M.G.; Dragila, M.I.; Kleber, M. Water uptake in biochars: The roles of porosity and hydrophobicity. *Biomass Bioenergy* 2014, 61, 196–205. [CrossRef]
- 49. Hazelton, P.; Murphy, B. Interpreting Soil Test Results. What Do All the Numbers Mean? 2nd ed.; CSIRO Publishing: Collingwood, Australia, 2007; ISBN 978 0 64309 225 9.
- 50. Cornelissen, G.; Jubaedah; Nurida, N.L.; Hale, S.E.; Martinsen, V.; Silvani, L.; Mulder, J. Fading positive effect of biochar on crop yield and soil acidity during five growth seasons in an Indonesian Ultisol. *Sci. Total Environ.* **2018**, *634*, 561–568. [CrossRef]
- 51. Crane-Droesch, A.; Abiven, S.; Jeffery, S.; Torn, M.S. Heterogeneous global crop yield response to biochar: A meta-regression analysis. *Environ. Res. Lett.* **2013**, *8*, 044049. [CrossRef]
- 52. Jeffery, S.; Verheijen, F.G.A.; van der Velde, M.; Bastos, A.C. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agric. Ecosyst. Environ.* **2011**, *144*, 175–187. [CrossRef]
- 53. Sohi, S.P.; Krull, E.; Lopez-Capel, E.; Bol, R. A Review of Biochar and Its Use and Function in Soil. *Adv. Agron.* 2010, 105, 47–82. [CrossRef]
- 54. Koide, R.T.; Nguyen, B.T.; Skinner, R.H.; Dell, C.J.; Peoples, M.S.; Adler, P.R.; Drohan, P.J. Biochar amendment of soil improves resilience to climate change. *GCB Bioenergy* **2015**, *7*, 1084–1091. [CrossRef]
- 55. Hardie, M.; Clothier, B.; Bound, S.; Oliver, G.; Close, D. Does biochar influence soil physical properties and soil water availability? *Plant Soil* **2014**, *376*, 347–361. [CrossRef]
- Peake, L.R.; Reid, B.J.; Tang, X. Quantifying the influence of biochar on the physical and hydrological properties of dissimilar soils. *Geoderma* 2014, 235, 182–190. [CrossRef]

- 57. Molnár, M.; Vaszita, E.; Farkas, É.; Ujaczki, É.; Fekete-Kertész, I.; Tolner, M.; Klebercz, O.; Kirchkeszner, C.; Gruiz, K.; Uzinger, N.; et al. Acidic sandy soil improvement with biochar—A microcosm study. *Sci. Total Environ.* **2016**, *563*, 855–865. [CrossRef]
- Sales, B.K.; Bryla, D.R.; Trippe, K.M.; Weiland, J.E.; Scagel, C.F.; Strik, B.C.; Sullivan, D.M. Amending Sandy Soil with Biochar Promotes Plant Growth and Root Colonization by Mycorrhizal Fungi in Highbush Blueberry. *HortScience* 2020, 55, 353–361. [CrossRef]
- 59. Van Zwieten, L.; Kimber, S.; Morris, S.; Chan, K.Y.; Downie, A.; Rust, J.; Joseph, S.; Cowie, A. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant Soil* **2010**, *327*, 235–246. [CrossRef]
- 60. Cross, A.; Sohi, S.P. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biol. Biochem.* **2011**, *43*, 2127–2134. [CrossRef]
- 61. Smith, J.L.; Collins, H.P.; Bailey, V.L. The effect of young biochar on soil respiration. *Soil Biol. Biochem.* **2010**, *42*, 2345–2347. [CrossRef]
- 62. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota–A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [CrossRef]