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RESEARCH ARTICLE

High-alkaline coconut shell biochar modifies nutrient availability-retention and enzymatic activity in calcareous soil: A rapid incubation soil test

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Abstract

Biochar (BC) is a solid by-product of the pyrolysis of organic matter and has several potential benefits. However, its high pH may also allow its use in calcareous soils. This study aimed to verify the impact of four doses (0, 10, 30 and 50 g·kg^{-1}) of coconut shell BC (pyrolyzed to 500 °C, pH 10.3) on the nutrient availability-retention and enzymatic activity of a calcareous soil with low fertility. The selected response variables were pH, electrical conductivity (EC), oxidation-reduction potential (Eh) of leachate and soil, phosphatase (PHO), arylsulfatase (ARY), fluorescein diacetate (FDA) activity and nutrient content in the soil and leachates. The experiment was carried out for 377 days under laboratory conditions in a pot test using calcareous soil (pH 8.03). In the leachates, EC decreased with the addition of 50 g·kg^{-1} BC. In the soil, an increase in Olsen-P (120 %), NO_3^- (42.5 %), K^+ (96.3 %) and Cu^{2+} (304.2 %) content was observed with 50 g·kg^{-1} BC. The 30 g·kg^{-1} BC treatment increased 47.5 % NO_3^-, 62.5 % K^+ and 137.5 % Cu^{2+} contents. BC reduced the leaching of NO_3^- (82 %), NH_4^+ (14.3 %), Olsen-P (21.8 %), Mg^{2+} (24.1 %), SO_4^{2-} (44.2 %), Ca^{2+} (18 %), Fe (33 %) and B (93 %). Finally, BC at 10 g·kg^{-1} increased ARY and FDA activities. The use of commercially manufactured coconut shell BC (30 and 50 g·kg^{-1}) is a viable alternative to improve nutrient availability in calcareous soils without significantly altering the naturally high pH of calcareous soils.

Keywords: arylsulfatase; fluorescein diacetate; ion availability; leaching; soil amendments

Introduction

Calcareous soils typical of arid and semi-arid regions exhibit extensive fertility restrictions due to low organic matter content (OMC), high pH, high carbonate ($CaCO_3^{2-}$) and/or bicarbonate (HCO_3^-) content, oxidative environments (Eh^+), potassium (K^+) and sodium (Na^+) accumulation, low nitrogen content and low phosphorus (P), iron (Fe), zinc (Zn), manganese (Mn), boron (B) and copper (Cu) availability. The loss of nutrients by leaching reduces the bioaccessibility of nutrients and plant productivity (1). However, calcareous soils have physical properties that are valuable and have excellent aggregate stability; thus, they have high productive potential if coupled with adequate water management, nutrient availability (1) and soil pH management. This can be mediated using organic amendments such as BC.

BC is obtained by thermochemical decomposition of organic biomass under minimal or no oxygen conditions at temperatures between 250 °C-750 °C (pyrolysis), which generates liquid (bio-oil), gaseous (smoke) and solid (biochar) by-products. The quality of these by-products is influenced by the pyrolysis conditions, temperature, time, speed, heating method and reaction environment (2). The solid by-product, BC, is generally produced at a high pH and can be used to promote the physicochemical properties of soils and improve their fertility. However, as this product is made from various biomass sources, its action in the soil varies (3).

Solid BC is characterized by high surface charge density, high surface area, high internal porosity, high levels of carbon, numerous functional groups, polar and non-polar surface sites, covalent bonds, van der Waals interactions, desorption microsites and ash content (3). These characteristics affect the capacity of biochar to retain and absorb the nutrient anions/cations. Likewise, these characteristics stimulate soil microbial communities (bacteria, fungi and microscopic fauna) and enzymatic activity (arylsulfatase, cellulase, dehydrogenase, invertase, phosphatase, urease and β-glucosidase) related to nutrient cycling by promoting labile carbon and volatile matter in the soil (4). Similarly, according to the characteristics of BC, it induces nutrient retention, mitigates the risk of leaching and promotes bioavailability of total nitrogen, P, K^+, Mn, Fe, Cu and Zn, favouring absorption by plants (4-6).

Although the treatment of soils with biochar has been widely studied in recent years, little research has been conducted on low-fertility, high-pH and low-OMC calcareous soils. Furthermore, farmers are concerned about the possible effects of BC, which generally has a high pH, on soil. Likewise, the process through which biochar is produced may cause variations in the pyrolysis temperature, which may reduce the biochar quality, resulting in unfavourable responses when used. This study hypothesizes that commercial high-alkaline BC modifies the characteristics of calcareous soil and improves its retention of nutrients without affecting soil pH. Considering that the standard application rates of BC in the field range from 1 to 100 $Mg \cdot ha^{-1}$, doses representing null, low, intermediate and high BC application were selected (3-5). In this context, the present study was performed to verify the impact of four doses (0, 10, 30 and 50 $g \cdot kg^{-1}$; corresponding to 0, 18.1, 54.4 and 90 $Mg \cdot ha^{-1}$, according to the soil characteristics) of commercially available high-alkaline biochar (coconut shells pyrolyzed to 500 °C, pH 10.3) on the nutrient availability-retention and enzymatic activity of a calcareous soil.

Materials and Methods

Biochar characteristics

Commercial BC was prepared from coconut shells and obtained by controlled pyrolysis carried out at a maximum temperature of 500 °C at a slow heating rate with an iodine number of 70 $mg \cdot g^{-1}$, Brunauer-Emmett-Teller (BET) surface area of 70 $m^2 \cdot g^{-1}$ and 2 % water solubility according to the manufacturer (Carbotecnia S.A. de C.V., Jalisco, Mexico). The BC samples were analyzed in a certified laboratory (NAPT-PAP-accredited, certification number ER-0223/2020, MAE: ISO 17025, ISO-9001:2015) (Table 1). Scanning electron microscopy (SEM) was also performed on the BC (Fig. 1) using a Philips XL30 ESEM equipped with an EDAX Genesis EDS system (SEMTech Solutions, Inc., North Billerica, MA, USA). The powder was placed in an aluminium holder using carbon conductive tape and coated with Au/Pd using a sputtering coater. The Frontier attenuated total reflectance (ATR) Fourier-Transform Infrared Spectrometer (FTIR) patterns were acquired using a Frontier FT-IR/NIR model 110711 (PIKE Technologies Inc., MA, USA) fitted with ATR MIRacle Diamond Frontier, in the range of 4000 cm^{-1} to 450 cm^{-1} wavenumber range with a resolution of 4 cm^{-1} (Fig. 2).

Soil column test

Calcareous topsoil samples (0-20 cm) were collected from rosetophilous soils in Saltillo, Coahuila, Mexico (25°21'14.5' N, -101° 2'25' W). After collection, the samples were air-dried, homogenised, sieved (2.5 mm diameter mesh) and characterised (Table 1). Subsequently, the experimental units were prepared by mixing 4.4 kg of air-dried soil with four quantities of biochar at 0, 10, 30 and 50 $g \cdot kg^{-1}$; these were designated as BC0, BC1, BC3 and BC5, respectively. The treatments were placed in a cylindrical PVC pipe with a height of 35 cm and an internal diameter of 10.5 cm. Then, a fine nylon mesh was placed at the bottom of each column using PVC coupling with a fine diameter and a height of 7.5 cm to prevent soil from entering the leachate. After mixing the soil with the BC and placing it in the PVC pipe, it was left to rest for seven days (stabilisation period). Subsequently, 100 mL of distilled water was added daily until leaching was observed. From this point, the soil moisture was kept constant using a tensiometer (Irrometer tensiometer LT 6") to maintain readings of 20 kPa (equivalent to 25 % gravimetric moisture). Throughout the study (377 days), the PVC tubes were maintained at room temperature (25 ± 5 °C). To verify the rapid, medium and prolonged effects of BC, soil and leachate samples were collected at 40, 140, 220, 310 and 377 days. Under field conditions, several factors (climate, soil type, crop and water management) can condition the response of BC and the results of this study can serve as a guideline for the behavior, potential effect and dynamics of this amendment in calcareous soils of low fertility.

Chemical traits of the soil and leachate

At 40, 140, 220, 310 and 377 days after the start of soil incubation, soil samples were collected at a depth of 10 cm. The soil was collected after leaching, air dried, homogenised and tested for pH and EC (HI98130 potentiometer, Hanna Instrument, Woonsocket, RI, USA) and oxidation-reduction potential, Eh (ORP-200 potentiometer, HM Digital Inc., Los Angeles, CA., USA) in a 1:2 ratio of soil to distilled water. For the leachate sample, the PVC soil column was placed in a plastic container, 100 mL of distilled water was added and 40 mL of leachate was collected within approximately 1 hr. Leachates were collected at 40, 140, 220, 310 and 377 days after the start of soil

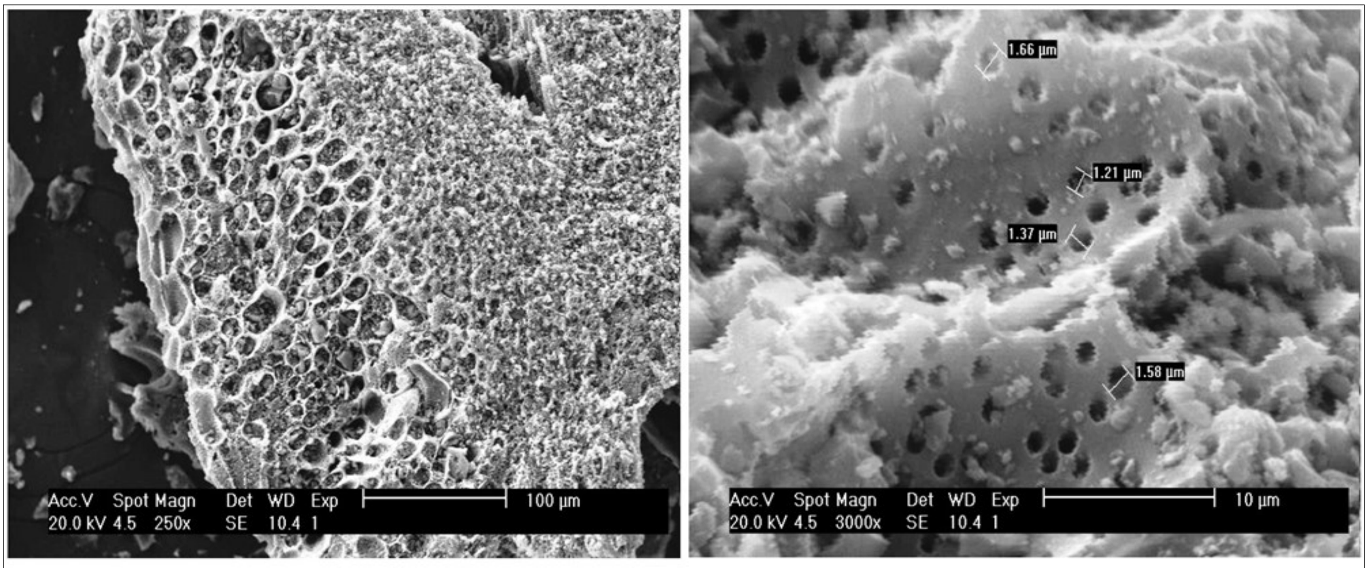

Fig. 1. Scanning electron microscopy (SEM) result of high-alkaline coconut shell biochar.

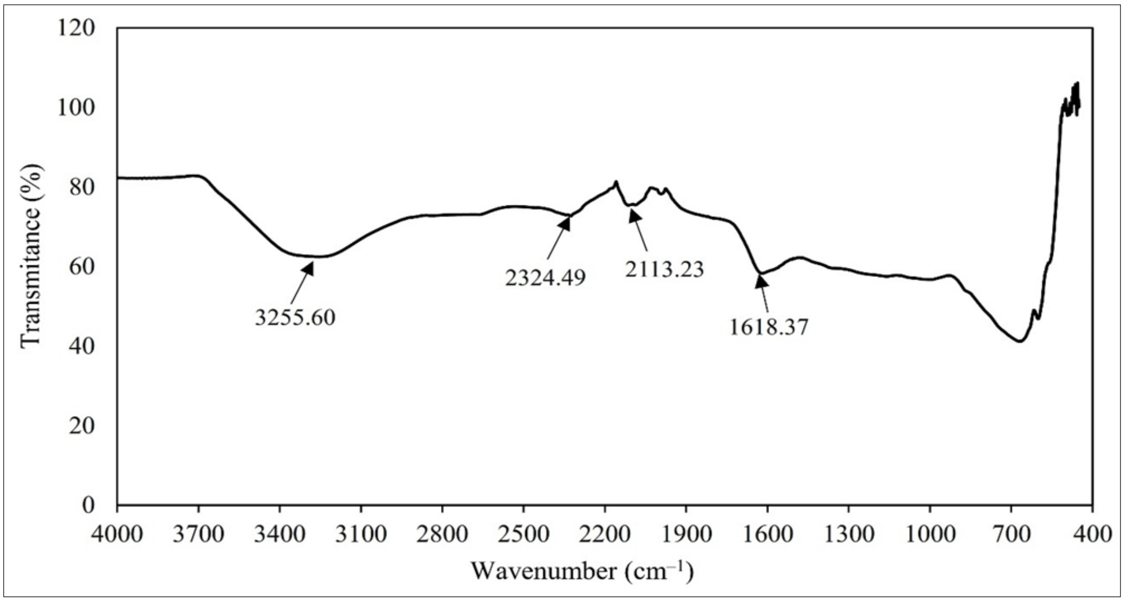

Fig. 2. Frontier attenuated total reflectance (ATR), Fourier-transform infrared spectrometer (FTIR) patterns of high-alkaline coconut shell biochar.

Table 1. Physicochemical characteristics of the coconut shell biochar and calcisol soil studied

Biochar		Soil	
Parameter	**Content**	**Parameter**	**Content**
Moisture (%)	3.34	Soil type	Loamy
Ash (%)	3.96	pH	8.03
Organic carbon (%)	55.7	EC ($mS{\cdot}cm^{-1}$)	1.13
OMC (%)	96	OMC (%)	0.99
C/N ratio	502	NO_3^- ($mg{\cdot}kg^{-1}$)	17.8
pH	10.3	Olsen-P ($mg{\cdot}kg^{-1}$)	28.0
EC ($mS{\cdot}cm^{-1}$)	4.3	K^+ ($mg{\cdot}kg^{-1}$)	477.0
Total N ($mg{\cdot}kg^{-1}$)	1.1	Ca^{2+} ($mg{\cdot}kg^{-1}$)	2,906
Olsen-P ($mg{\cdot}kg^{-1}$)	0.6	Mg^{2+} ($mg{\cdot}kg^{-1}$)	268
K^+ ($mg{\cdot}kg^{-1}$)	7.4	SO_4^{2-} ($mg{\cdot}kg^{-1}$)	32.5
Ca^{2+} ($mg{\cdot}kg^{-1}$)	1.4	Na^+ ($mg{\cdot}kg^{-1}$)	28.0
Mg^{2+} ($mg{\cdot}kg^{-1}$)	0.4	B ($mg{\cdot}kg^{-1}$)	0.53
SO_4^{2-} ($mg{\cdot}kg^{-1}$)	0.3	Cu ($mg{\cdot}kg^{-1}$)	0.30
Na^+ ($mg{\cdot}kg^{-1}$)	1.1	Fe ($mg{\cdot}kg^{-1}$)	2.04
B ($mg{\cdot}kg^{-1}$)	14.9	Mn ($mg{\cdot}kg^{-1}$)	3.05
Cu ($mg{\cdot}kg^{-1}$)	51.73	Zn ($mg{\cdot}kg^{-1}$)	1.67
Fe ($mg{\cdot}kg^{-1}$)	657.0		
Mn ($mg{\cdot}kg^{-1}$)	36.07		
Zn ($mg{\cdot}kg^{-1}$)	24.05		

incubation. The pH, EC and Eh were measured *in situ* immediately after obtaining leachate (10 mL) from the PVC column. Subsequently, the leachate was stored in a plastic container and placed at 4 °C.

Soil and leachate nutrient content

At the end of the experiment, the soil from the PVC pipe was air-dried, homogenised and sieved. A 200 g sample of each replicate per treatment was mixed homogeneously to generate a composite sample per treatment (with three replicates). The stored leachate (4 ° C) from each treatment was mixed to form a composite sample per treatment (with three replicates), which was used to determine the accumulated mineral concentrations of the leachates at the end of the experiment. Mineral concentrations were determined in a certified laboratory (7).

Enzymatic activity

Soil samples were taken at 40, 140, 220, 310 and 377 days after the start of soil incubation. Samples were collected at a depth of 10 cm. After obtaining the leachate, it was air-dried for one hour to remove excess water and stored at 4 °C (8). PHO activity was determined by colourimetric estimation of the released p-nitrophenol (9). ARY was quantified according to earlier research (10) and FDA (3′, 6′-diacetyl-fluorescein) by hydrolysis (11).

Statistical analysis

Data on leachates, soil and enzymatic activity were subjected to a Shapiro-Wilk normality test. Levene's test was performed to verify the homoscedasticity of the data. Subsequently, data were analysed using repeated-measures multivariate analysis of variance (RM-MANOVA), followed by a multiple means comparison test (Fisher least squares difference). The nutrient content (soil and leachates) was analysed using analysis of variance (ANOVA) followed by a multiple means comparison test (Fisher least squares difference). Data analysis was carried out considering $p < 0.05$, using the IBM SPSS v19 program.

Results and Discussion

The SEM image of BC shows an amorphous surface as well as "cavernous" spaces (Fig. 1). Many well-defined micropores (< 2 µm) are evident within the folds of the carbonaceous material. Derived from the porous structure of carbonaceous material, it has a large surface area (BET surface area of 70 $m^2{\cdot}g^{-1}$), which affects soil characteristics and properties. FTIR analysis showed the presence of the fingerprint region (600-1500 cm^{-1}), double bond region (1500-2000 cm^{-1}), triple bond region (2000-2500 cm^{-1}) and single bond region (2500-4000 cm^{-1}), revealing several peaks at 3255.60 cm^{-1}, 2324.49 cm^{-1}, 2113.23 cm^{-1} and 1618.37 cm^{-1}, indicating the complex molecular architecture and reactive nature of this carbonaceous material (Fig. 2).

Soil chemical traits

The soil variable data presented a normal distribution ($p > 0.05$), homogeneity of variances ($p > 0.05$) and significant differences ($p < 0.05$, RM-MANOVA). For the soil parameters, significant differences ($p < 0.05$) were found between the sampling times; the pH showed the highest value (8.36 with BC0) at 40 days, while the lowest value (7.87) was documented at 220 days under the BC5 treatment. The EC was highest (0.22 $mS{\cdot}cm^{-1}$) at 40 days and lowest (0.10 $mS{\cdot}cm^{-1}$) at 140 days. Finally, Eh was highest (71.0 mV, with BC5) at 377 days and lowest (35.6 mV) at 40 days under BC5 treatment.

Differences between treatments were observed in pH, EC and Eh ($p < 0.05$). The soil pH decreased between treatments with the addition of BC5 and BC3, resulting in the lowest values. The most significant decrease was observed at 40 and 377 days in these treatments, with a reduction of 3.44 % and 2.21 %, respectively. For EC, mean values between 0.10 and 0.22 $mS{\cdot}cm^{-1}$ were documented and an increase was observed at 40, 220 and 310 days (by 29.4 %, 4.8 % and 7.7 %, respectively) in treatment BC3 and an increase by 0.01 units was observed at days 310 and 37 under BC5 treatment, compared to BC0. However, for BC1, there was a decrease of 35 % at 377 days. For soil Eh, it was possible to appreciate a decrease in the values at 40 days under all treatments before a subsequent increase; the BC3 treatment presented the highest readings with respect to BC0 at 40 and 330 days (Table 2). At the end of the soil experiment, a statistical reduction in pH (by 0.10, 0.18 and 0.25 units) was found in treatments BC1, BC3 and BC5, respectively, compared to BC0. Regarding the EC, we observed a decrease compared to BC0 by 0.06, 0.04 and 0.01 units in BC1, BC3 and BC5, respectively. The redox potential increased significantly in BC3 and BC5 (by 5.0 and 10.4 mV, with the control).

Leachate chemical traits

All data presented a normal distribution ($p > 0.05$) and homogeneity of variance ($p > 0.05$). For the pH, EC and Eh of the leachates, significant differences were observed at different sampling times (RM-MANOVA, $p < 0.05$). For the different samplings, it was found that at 220 and 310 days, the highest pH values were found in leachates, 8.77 and 8.64, respectively in BC5 ($p < 0.05$). The EC of leachates was highest at 310 days (with BC0), whereas the lowest mean values were found at 377 days. Eh showed the lowest values at 140 days (BC3 and BC5), whereas at 220 days (with BC1 and BC3) and 377 days (with BC3), it presented the highest values ($p < 0.05$).

For the different doses of biochar used, it was observed that the pH increased in all treatments throughout the incubation period and the lowest value was observed at 140 days under treatment BC5, whereas the highest value was observed under BC5 at 220 days (pH 8.77). The EC showed a decreasing trend under treatment BC5; it should be noted that at 310 days, there was an increase in EC values, which then declined until the end of the incubation period. Owing to the oxide-reducing capacity (redox pair) of the ions dissolved in the leachates, the Eh values oscillated between 90 and 175 mV. The lowest values were observed at 140 days, with BC3 being the biochar dose that induced the lowest values in the leachates compared to BC0.

Soil nutrient concentration

An increase by up to 120 % was found in the Olsen-P content under the application of BC5 compared to BC0. The content of NO_3^- increased by 47.5 % and 42.5 % with the application of BC3 and BC5. For K^+, a substantial increase of 62.5 % and 96.3 % was documented in treatments BC3 and BC5, respectively, compared to BC0. The addition of BC3 increased SO_4^{2-} in the soil by 186.4 %, whereas it was reduced in BC5 by 85.6 % compared to that in BC0. The Na^+ content increased with the addition of biochar, with treatment BC5 showing the highest value of 47.1 $mg{\cdot}kg^{-1}$, an increase of 164.6 % compared to BC0. Among the micronutrients, Cu showed a higher concentration

Table 2. Chemical properties of the soil and leachates of calcisol soil added with coconut shell biochar

T	Incubation period (days)	Soil			Leachates		
		pH	EC ($mS{\cdot}cm^{-1}$)	Eh (mV)	pH	EC ($mS{\cdot}cm^{-1}$)	Eh (mV)
BC0	40	8.36±0.11^{a†}	0.17±0.04^{b}	41.4±2.51^{a}	7.91±0.12^{b}	1.55±0.30^{b}	117±6.22^{a}
	140	8.07±0.05^{a}	0.14±0.01^{a}	36.4±1.52^{c}	8.25±0.10^{a}	1.18±0.26^{b}	104±2.05^{a}
	220	8.03±0.04^{a}	0.21±0.01^{a}	55.2±1.64^{b}	8.51±0.07^{b}	0.96±0.01^{c}	170±4.39^{ab}
	310	8.13±0.06^{a}	0.13±0.02^{a}	56.4±2.07^{d}	8.55±0.06^{a}	2.23±0.77^{a}	152±5.43^{c}
	377	8.15±0.05^{a}	0.17±0.01^{a}	60.6±2.07^{c}	8.42±0.04^{a}	0.78±0.14^{b}	169±2.70^{b}
BC1	40	8.11±0.05^{b}	0.13±0.02^{b}	14.8±2.95^{c}	8.02±0.07^{ab}	1.42±0.21^{b}	116±5.89^{a}
	140	8.11±0.01^{a}	0.10±0.01^{c}	37.4±0.55^{c}	8.22±0.04^{a}	1.03±0.09^{b}	103±1.41^{a}
	220	7.99±0.02^{a}	0.17±0.01^{b}	55.6±1.14^{b}	8.49±0.03^{b}	1.03±0.03^{b}	173±3.83^{a}
	310	7.97±0.07^{b}	0.13±0.01^{a}	60.2±1.30^{c}	8.55±0.08^{a}	1.13±0.18^{b}	157±3.36^{ab}
	377	8.05±0.04^{b}	0.11±0.01^{c}	60.6±1.82^{c}	8.36±0.04^{a}	0.73±0.11^{b}	171±1.92^{b}
BC3	40	7.92±0.03^{c}	0.22±0.01^{a}	36.2±0.84^{b}	8.05±0.04^{a}	2.05±0.28^{a}	113±2.88^{a}
	140	7.99±0.01^{b}	0.13±0.01^{a}	46.6±1.95^{b}	8.15±0.09^{a}	1.68±0.31^{a}	90±10.17^{a}
	220	7.89±0.07^{b}	0.22±0.03^{a}	59.8±2.05^{a}	8.57±0.11^{b}	1.48±0.07^{a}	174±2.49^{a}
	310	7.98±0.02^{b}	0.14±0.01^{a}	62.6±0.89^{b}	8.57±0.07^{a}	2.03±0.35^{a}	160±4.15^{a}
	377	7.97±0.02^{c}	0.13±0.01^{bc}	65.6±0.55^{b}	8.40±0.06^{a}	1.09±0.14^{a}	175±1.30^{a}
BC5	40	8.00±0.07^{c}	0.12±0.02^{b}	35.6±3.85^{b}	8.06±0.08^{a}	2.04±0.14^{a}	98±2.95^{b}
	140	7.95±0.02^{c}	0.11±0.00^{b}	50.8±0.45^{a}	8.00±0.05^{b}	1.37±0.36^{a}	92±21.98^{a}
	220	7.91±0.04^{b}	0.15±0.02^{b}	60.0±1.22^{a}	8.77±0.04^{a}	1.00±0.02^{bc}	168±2.07^{b}
	310	7.91±0.03^{b}	0.14±0.01^{a}	65.8±0.45^{a}	8.64±0.11^{a}	1.15±0.28^{b}	152±2.61^{bc}
	377	7.87±0.03^{d}	0.16±0.01^{ab}	71.0±1.87^{a}	8.46±0.14^{a}	0.87±0.16^{b}	168±3.90^{b}
T		< 0.0001	< 0.0001	< 0.0001	0.0838	< 0.0001	< 0.0001
S		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001
T × S		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0020

T = treatment, S = sampling, EC = electrical conductivity, Eh = oxidation-reduction potential, n = 5, † = distinct letters in the row indicate significant differences according to LSD's test ($p < 0.05$). Means ± standard deviation.

with biochar application: 45.8 % (BC1), 137.5 % (BC3) and 304.2 % (BC5) more than with BC0. In the case of B and Zn, the addition of BC3 induced the highest values (0.45 and 1.29 mg·kg^{-1}, respectively). However, Mn was higher with the addition of BC1 (10.6 % more than BC0) but dramatically reduced (by 59.3 %) with the application of BC5 (Table 3).

Leachate nutrient concentration

The application of BC1, BC3 and BC5 reduced the NO_3^- content in the leachate by 82 %, 75 % and 42 %, respectively, while BC1 and BC3 showed a lower content of NH_4^+ compared to BC0. The Olsen-P content decreased by 21.8 % in BC1 and BC5 and the contents of K^+, Ca^{2+} and SO_4^{2-} increased in BC3 (by 82 %, 20.2 % and 68.8 %, respectively) compared to BC0. Similarly, K^+ increased by 69.8 % in BC5. In the leachate from column BC1, lower contents of Mg^{2+} and SO_4^{2-} (24.1 % and 44.2 %, respectively) were observed and the leachate from BC5 showed an 18 % reduction in Ca^{2+} compared to BC0. Alternatively, for the BC3 and BC5 treatments, there was a notable increase in Na^+ (127 % and 162 %, respectively). All treatments increased the Cl^- concentration. For BC3, there was a 37.9 % increase in the total carbonate (HCO_3^-) content compared to the control values. Regarding micronutrients, Fe was reduced by 33 % in the accumulated leachate when biochar was applied. Likewise, B was reduced by 67 % and 93 % when BC1 and BC3, respectively, were applied, whereas for the leachate obtained from treatment BC5, B was not detected with respect to BC0. Regarding Mo, when compared to the control treatment, an increase of 400 % and 233.3 % was observed in BC1 and BC5, respectively. Zn content increased by 200 % for all biochar applications (Table 3).

Soil enzymatic activity

According to RM-MANOVA, the enzymatic activity of ARY and the total microbial activity (FDA hydrolysis) were significantly different ($p < 0.05$). Concerning ARY activity, values between 121 and 583 µg of p-nitrophenol released g^{-1} soil·h^{-1} were found, presenting a maximum reduction at 220 days in all treatments. The addition of BC1 increased the activity of this enzyme by 180 % and 97.6 % compared to that of BC0 at 220 and 310 days, respectively, representing the highest ARY activity. Moreover, at the end of the incubation period, the highest ARY activity was observed under BC3 treatment (19.7 % more than that of BC0). A 28.9 % reduction was observed with the addition of BC5 (Table 4). FDA activity (total microbial activity) exhibited significant fluctuations after 140 days; here, BC5 was statistically similar to BC0, while BC1 and BC3 reduced activity by 82 % and 51 %, respectively, compared to the control. By 220 days, BC1 and BC3 treatments significantly ($p < 0.05$) increased fluorescein activity compared with the control. Similarly, at 310 days, treatments BC1 and BC5 showed a significant increase in this parameter. In the last sampling, B5 induced a 115 % increase in activity compared to BC0 (Table 4). The variations in enzyme activity documented in this study likely reflect the temporal behaviour of the diversity, richness and balance of the soil and rhizosphere microbial community due to the addition and maturation process of BC, which is associated with the data obtained from the FDA.

The capillary surface structure of biochar varies depending on the pyrolysis temperature; less than 400 °C generates macropores, whereas 500-600 °C generates micropores in response to the generation of volatile substances (12). The high level of porosity presented by biochar (Fig. 1) increases its total surface area, making it a potential habitat for microorganisms and thus improving moisture retention and mineral absorption (12). The FTIR peaks may be the result of the properties and curvatures of the cellulose and hemicellulose fibres of the coconut shells subjected to pyrolysis at 500 °C. These peaks (Fig. 2) are associated with the presence of alcohols, phenols and hydroxyl compounds (O-H stretching) and $-NH_2$ group (3255.60 cm^{-1}), C-H (2324.49 cm^{-1}), alkyne group (C=C stretching) (2113.23 cm^{-1}) and alkene and cyclic alkene groups (C=C stretch) (1618.37 cm^{-1}), which denote the complex molecular architecture and reactive nature of this carbonaceous material (13). Such peaks may be due to the curvatures of the plant material fibres. SEM images and FTIR analysis can be used to characterise biochar to establish its potential use in different soil types.

There was no substantial increase in soil pH during the incubation period with the addition of alkaline BC. On the contrary, research indicates that an increase in the pH of calcareous soil by up to 0.20 units (4). The different results obtained here may be due to the oxidation processes of the C contained in the biochar, which originated from acidic carboxyl groups during the incubation period and the doses used. Surprisingly, the BC used (highly alkaline, pH=10.3) did not significantly increase the pH of the calcareous soil (14). This phenomenon can be explained by the fact that, during the

Table 3. Effect of coconut shell biochar on average soil nutrient and leachate concentration

Parameter	Soil				Leachates			
	BC0	BC1	BC3	BC5	BC0	BC1	BC3	BC5
CEC [cmol(+) kg^{-1}]	16.0^{a†}	16.5^a	17.5^a	17.1^a	-	-	-	-
Alkalinity	6.70^{a*}	7.09^{a*}	7.21^{a*}	5.81^{a*}	174^{c**}	185^{c**}	240^{a**}	219^{b**}
NO_3^- (mg·kg^{-1})	7.93^b	8.92^b	11.70^a	11.30^a	61.30^a	35.40^b	10.90^c	15.30^c
NH_4^- (mg·kg^{-1})	nd	nd	nd	nd	6.76^a	5.79^b	5.79^b	6.28^{ab}
Olsen-P (mg·kg^{-1})	40.10^b	39.40^b	41.80^b	88.40^a	3.24^a	2.53^b	2.83^b	2.53^b
K^+ (mg·kg^{-1})	325.0^c	383.0^c	528.0^b	638.0^a	55.50^b	60.6^b	101.0^a	93.4^a
Ca^{2+} (mg·kg^{-1})	2,856^a	2,922^a	3,015^a	2,861^a	178^b	160^{bc}	214^a	146^c
Mg^{2+} (mg·kg^{-1})	313.0^a	293.0^a	267.0^a	304.0^a	53.8^a	40.8^b	55.8^a	49.5^{ab}
SO_4^{2-} (mg·kg^{-1})	30.9^b	26.58^b	88.50^a	4.44^c	199.00^b	111.00^c	336.00^a	180.00^b
Na^+ (mg·kg^{-1})	17.80^c	18.30^c	34.90^b	47.10^a	22.30^c	23.90^c	50.60^b	58.40^a
Cl^- (mg·kg^{-1})	^{nc}	^{nc}	^{nc}	^{nc}	65.1^c	149.0^b	139.0^b	184.0^a
B (mg·kg^{-1})	0.35^a	0.35^a	0.45^a	0.39^a	0.150^a	0.050^b	0.010^c	0.000^c
Cu (mg·kg^{-1})	0.24^c	0.35^{bc}	0.57^b	0.97^a	0.0007^a	0.0008^a	0.0008^a	0.0008^a
Fe (mg·kg^{-1})	1.75^a	1.68^a	1.59^a	1.58^a	0.003^a	0.002^b	0.002^b	0.002^b
Mn (mg·kg^{-1})	1.23^a	1.36^a	1.26^a	0.50^b	0.002^a	0.002^a	0.002^a	0.002^a
Mo (mg·kg^{-1})	^{nc}	^{nc}	^{nc}	^{nc}	0.0006^c	0.003^a	0.001^c	0.002^b
Zn (mg·kg^{-1})	0.90^b	0.79^b	1.29^a	0.86^b	0.001^b	0.003^a	0.003^a	0.003^a

†= distinct letters in the row indicate significant differences according to LSD's test ($p < 0.05$). ^{nc} = unquantified; nd = undetected; * = total carbonates (%), ** = bicarbonates (HCO_3^- as mg·kg^{-1}), CEC = cation exchange capacity.

initial phases, some fractions of non-aromatic C from BC may be oxidised, which reduces the soil pH (15, 16). Likewise, nitrification mechanisms, NH_4^+ retention and the presence, release and mineralisation of functional groups and low-molecular-weight humic compounds in BC induce an acidic environment that prevents a substantial increase in the pH of calcareous soil (4, 17, 18). Interestingly, these phenomena could persist regardless of the short-term maturation process of BC in the soil, therefore we believe that the BC × calcareous soil interaction could create a "pH buffer zone." This is supported by the difference between the soil pH (before adding BC) and the BC pH, which in our case was 2.27 units (Table 1) (19). Fluctuations in soil pH throughout the incubation period may indicate the release, oxidation and/or mineralisation kinetics of BC functional groups in the soil (4, 15, 16, 18) within the suggested buffer zone. Alternatively, the increase observed in BC5 may result from modifications in the EC of the soil due to the ash content of the biochar (3.96 %) as well as the soluble alkaline elements released, K^+, Ca^{2+} and Na (4, 14). The alterations in the redox potential observed with BC may be due to the release of some functional groups from the biochar surface (mainly quinone, phenol, carboxyl, hydroxyl, carbonyl and aromatic ring residues) upon interaction with soil particles or by the generation of an organomineral layer on the soil surface by electron transfer between biochar and soil minerals (20).

The chemical traits of leachates are indicative of soil quality and the potential content of dissolved ions; fluctuations in leachate pH may be due to the decarboxylation processes of organic anions, proton consumption and the high carbonate content present in the soil (21). Conversely, the decrease in EC due to the addition of BC1 may be the result of the ability of the treatment to absorb and retain mineral nutrients in its surface layers, which prevents leaching loss and a probable reduction in soluble salts (21). The trend in Eh observed in the results could be caused by the constant processes of losing and gaining electrons in the soil-biochar system (20).

The increase in inorganic soil nitrogen observed in our study was similar when 2 % maize BC (pyrolysed at 200 °C to a calcareous soil; pH 7.7) and 5 and 10 $g{\cdot}kg^{-1}$ of maize BC (pyrolysed at 200 °C, 400 ° C and 600 °C in Haplocalcid typic soil; pH 8.03) were used (4, 14). The increase in NO_3^- content in the soil and the low concentration in leachates possibly stems from adsorption on the surface of the biochar as well as increased microbial activity and an increase in water retention (22-24) or by increased mineralization and nitrification processes in the soil caused by the leaching events that occurred during the incubation period (25-27). This reduction minimized the amount of nitrate pollution in the groundwater. Research indicates that a reduction of 80.16 % in the accumulated leaching of ammoniacal nitrogen with applications of 2 % BC (400 °C) in soil with a pH of 7.9 (23).

The reduction in soil pH (Table 2) could create a favourable environment for phosphorus dissolution, thus, the increase in phosphorus in soil may be due to electrostatic interactions that occur with the hydroxyl/carboxyl groups of the BC, releasing the Olsen-P from mineral precipitates with Ca^{2+}, Fe^{3+} and oxyhydroxides present in calcisol soil (21, 28). This bioavailable phosphorus can be captured or absorbed by complexation on the BC surface, reducing its mobility (29, 30), due to the presence of oxonium and pyridinium groups and protonated aromatic structures that could increase the anion-exchange capacity (28). The increase in K^+ and SO_4^{2-} contents in the soil by BC can be explained by the increase in FDA activity (Table 4), which excited the activity of K^+ in solubilising microorganisms and promoted the transformation of organic sulphur to sulphate under different soil conditions (27). Similar results were obtained when 5 and 10 $g{\cdot}kg^{-1}$ BC from corn residues (pyrolyzed at 200 °C, 400 °C and 600 °C) and with BC from wood chips (*Pinus pinastere* and *Pinus radidata*) were obtained by gasification at 600 °C-900 °C and at a dose of 30 $Mg{\cdot}ha^{-1}$ on Calcixerept typic loam soil (pH 8.3) (14, 31). The high K^+ content in the BC used induces a high release rate into the soil pore water, causing its leaching (4, 5). The soil Na^+ content (increased by BC5) did not pose a serious risk to soil salinisation. Moreover, as explained above, this mineral was lost via leaching.

In the case of micronutrients, one partial explanation for their increase in the soil is their presence in the BC used (Table 1). The increase in Zn content is similar when applying 2 % pyrolysed BC at 200 °C on calcisol soil with a pH of 7.7. However, the high Zn

Table 4. Effect of coconut shell biochar on the enzymatic activity of calcareous soil

T	Incubation period (days)	Phosphatase activity (µg p-	Arylsulfatase activity (µg p-	Fluorescein diacetate hydrolysis
BC0	40	630±75^{a†}	482±122^{a}	5.6±1.2^{b}
	140	806±154^{a}	445±83^{a}	18.6±4.5^{a}
	220	891±135^{a}	121±17^{b}	0.4±0.1^{c}
	310	618±80^{a}	255±77^{b}	0.3±0.1^{b}
	377	731±139^{a}	487±42^{a}	8.0±2.1^{b}
BC1	40	482±118^{a}	270±107^{a}	7.6±1.3^{ab}
	140	735±116^{a}	416±107^{a}	3.3±1.3^{b}
	220	706±137^{a}	339±47^{a}	19.1±2.1^{a}
	310	744±200^{a}	504±69^{a}	6.9±3.1^{a}
	377	749±91^{a}	494±62^{a}	4.3±0.3^{bc}
BC3	40	633±83^{a}	447±107^{a}	4.7±0.2^{b}
	140	866±116^{a}	428±116^{a}	9.1±1.6^{b}
	220	647±141^{a}	131±37^{b}	12.2±3.2^{b}
	310	699±153^{a}	285±65^{b}	1.4±0.3^{b}
	377	530±65^{a}	583±31^{a}	0.3±0.1^{c}
BC5	40	695±138^{a}	414±131^{a}	11.9±3.2^{a}
	140	877±139^{a}	429±96^{a}	11.6±2.9^{ab}
	220	942±106^{a}	256±81^{ab}	2.4±0.5^{c}
	310	825±137^{a}	354±35^{ab}	6.1±0.2^{a}
	377	674±139^{a}	346±6^{b}	17.2±1.6^{a}
T		0. 3674	0.7815	0.0050
S		0.1207	0.0001	0.0001
T × S		0.9046	0.1324	<0.0001

† = distinct letters in the row indicate significant differences according to LSD's test ($p < 0.05$). T = treatment, S = sampling. n = 5. Means ± standard deviation

content accumulated in leachates may be due to the dissolution of this nutrient from BC into the soil solution (32). The increase in Cu, B, Zn and Mn may be associated with a decrease in soil pH (Table 2) associated with the increase in microbial activity (Table 4). At the same time, B and Fe could precipitate or adsorb on soil particles because of the formation of Fe hydroxides by redox reactions on the BC surface, avoiding their leaching, remembering that these nutrients are particularly sensitive to changes in pH (33). It is worth mentioning that soil nutrient losses depend on the BC type, application rate, soil type and soil depth and the release and leaching of some nutrients also depend on soil wetting events (disaggregation) and desiccation (water loss by drainage, evaporation and plant uptake) (22, 29, 34, 35). Thus, the addition of BC reduces nutrient loss by leaching as a result of an improvement in the soil-BC-water ratio, its large surface area, high porosity, polar and non-polar groups and the presence of carboxyls and hydroxyls on its surface (34, 36).

Finally, the increase in ARY activity with the addition of BC may be because it improves the forms of sulphur present in the soil and probably the generation of SO_4^{2-} sorption sites, in addition to exacerbating the soil sulphur-microbial biomass involved in sulphur transformation (37). Likewise, BC creates a microenvironment that reduces and/or prevents the degradation or denaturation of soil enzymes and the large number of meso-and micropores, the presence of accessible volatile compounds and the contribution of labile carbon skeletons can increase the soil microbial load (4, 23, 38). Our results are consistent with this hypothesis, as an increase in FDA hydrolysis activity was found with BC. Research indicates that biochar increases microbial activity in calcisol soils with a very low OMC (< 1.0 %) (14). However, the fluctuations observed in FDA hydrolysis activity during the incubation period may be due to BC maturation processes or the dynamics of soil wetting-drying processes, redox environments or soil pH (3, 21, 39, 40). Similarly, they can affect physicochemical processes, biomass mineralisation, soil nutrient content and ion biogeochemistry (26, 35).

Conclusion

Our soil incubation results suggest that the application of commercial coconut shell biochar at doses of 30 and 50 $g{\cdot}kg^{-1}$ in calcareous soil improves nutrient availability, reduces nutrient loss by leaching and increases ARY and FDA hydrolysis activity without raising the pH level. The use of biochar can be implemented in low-fertility calcareous soils, however, the biochar production cost must be considered when evaluating its potential as an accessible and sustainable application in this type of soil under field conditions. By following certain guidelines to make pyrolysis more efficient, farmers can locally produce biochar from waste from their agroecosystems, which would reduce the cost of purchasing it (US$1.48 per kg-cost of biochar used here). However, it is recommended to carry out soil incubation tests and soil fertility tests and plan the crops to be grown to determine the ideal application rate of biochar.

Authors' contributions

MCLP carried out the investigation and co-wrote the original draft. AJM participated in the study design and performed statistical analysis. ABM coordinated the study and contributed to its organization. EGL was involved in study coordination and management. FPL conceptualized the investigation, contributed to study design and co-wrote the original draft. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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