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Influence of biochar derived from lignin-rich feedstocks on soil properties and crop yield: the Case of *Solanum lycopersicum* L. (tomatoes)

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Abstract

A sustainable pathway for valorizing the growing volume of lignin-rich organic feedstocks (LRFs) in emerging economies is to convert them into biochar to sequester carbon and improve soil fertility. However, biochar derived from such LRF may not always show favorable outcomes during soil application. Their interaction with the soil–plant–microbial ecosystem is very complex, and comparative investigations with other common types of biochars are lacking. This study investigated the impact of walnut shell biochar (WSB) and wood chip biochar (WCB) on soil biological properties and crop yield during the growth of *Solanum lycopersicum* L., and compared them with those of plant waste biochar (PWB) derived from agricultural plant residues. Among biochar variants, only PWB (1% w/w) has increased tomato yield compared to unamended soil. It also showed better carbon mineralization that stemmed from its higher degradability, lower carbon content, and higher H/C ratio. In contrast, WSB showed a relatively higher hydrophobicity, carbonization, and aromaticity that reduced its accessibility to soil microorganisms. Despite these characteristics and higher ash content, WSB did not lower soil enzyme and microbial activity, unlike WCB. At the same time, WSB did not improve crop yield. Mixing WSB (3% w/w) with soil has slightly enhanced the soil carbon stabilization. The high carbon recalcitrance of LRF may necessitate longer aging in soil compared with PWB to showcase any measurable (short/long) benefits to crop yield and soil characteristics.

Introduction

The transformation of refuse biomass to biochar for soil storage can facilitate the value-added recycling of organic waste by concurrently sequestering carbon and improving soil health. The type of feedstock greatly influences the physicochemical properties of biochar, such as porosity, water holding capacity, pH, and its effects on soil biota (Godlewska, Ok and Oleszczuk, 2021), thereby dictating its soil application potential and overall efficacy. For example, biochar from the organic fraction of municipal solid waste and sewage sludge is beneficial for the detoxication of various contaminants and pollutant immobilization (Krahn et al., 2023; Zhao et al., 2023) but less viable for agricultural application (Goldan et al., 2022), due to the risk of intrinsic potentially toxic elements (PTEs) such as heavy metals. On the contrary, biochar derived from agricultural waste (Sulok et al., 2021) or food waste (Palansooriya et al., 2023) can be designed to contain minimal PTEs (Wang et al., 2023). This improves their soil applicability for growing edible crops (Pandey et al., 2022; Tiong et al., 2024) and feed for livestock (Kana et al., 2011). Thus, the most widely used biochar feedstocks for soil application are agricultural and plant wastes, such as rice husk, switchgrass, and fruit processing wastes.

Lignin-rich organic feedstocks (LRFs), such as wood chips and walnut shells, are also an abundant residue from agriculture. For instance, Europe is one of the largest importers of shelled walnuts, with emerging countries, such as India, Chile, and Ukraine, being the largest suppliers (CBI, 2019). Even those walnuts cultivated in European countries, such as France, are sent to locations, such as Moldova, for shelling due to the high domestic processing/shelling costs. Thus, these emerging economies are left with large quantities of residual shells, which are usually burned for disposal. Their transformation to biochar presents a sustainable valorization pathway that can sequester carbon and can also economically support the local farmers if the biochar can also be used for soil amendment. LRFs contain fewer intrinsic PTEs, and the biochar derived from them has enhanced pore surface area, stability, and lower ash content (Adhikari, Timms and

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Mahmud, 2022). However, depending on the extent of carbonization, the presence of nutrients, and the type of soil, they may not show favorable benefits to crop fertility.

Walnut shell biochar (WSB) prepared at 600 °C has been shown to lower N_2O emissions from the soil (Sial et al., 2022), while that at the highest treatment temperature (HTT) of 500 °C was promising for the remediation of Cd-contaminated soil (Qiu et al., 2018). The existing tests with WSB have highlighted positive effects of WSB, such as improved soil properties (retention of exchangeable cations such as Ca^{2+} and K^+) (Suddick and Six, 2013), increased crop yields (wheat and lentil) in the first and second years during a 4-year period (Safaei Khorram et al., 2020), and enhanced soil aggregation that facilitated long-term stabilization of soil organic matter (SOM; Wang et al., 2017). However, on the other hand, WSB (applied at an HTT of 600 °C) application has also demonstrated increasing CO_2 emissions relative to the soil control (during the first 70 days) (Sial et al., 2022), no changes to bamboo root growth (Wang et al., 2019), and waning influence on tomato fruit and corn yield after the second year in a 4-year field test (Griffin et al., 2017). Similarly, wood chip biochar (WCB) produced at 500 °C has been shown to improve soil-C sequestration without much benefits to plant growth (Gonzaga et al., 2018), while another investigation noticed an improvement in the crop yield of winter wheat (Wyzińska, Berbeć and Grabiński, 2023). A 3-year field application highlighted the benefits of adding WCB prepared at 450 °C to the plant performance of maize (Jones et al., 2012). However, the addition of WCB that was prepared between 600 and 900 °C on an alkaline soil did not improve nutrient uptake or crop yield in a Mediterranean agroecosystem (Martos et al., 2019). In short, the interaction between soil, plants, and microbes is a complex, intricate, and dynamic relationship and depends significantly on the type of added biochar and the extent of carbonization. Comparative investigations of the soil application viability of biochar prepared from lignin-rich feedstocks are currently limited.

In this study, the research question was to investigate whether the WSB (from LRF) produced in-house has the potential benefit as a soil improver compared to the commercially available biochar, WCB (from LRF), and plant waste biochar (PWB) (from non-LRF), during their early stages of application in the soil. For this, a 100-day pot experiment using tomato plants (*Solanum lycopersicum* L.) was conducted to assess the effects of WSB and WCB on soil properties and crop yield in comparison to that of PWB. Herein, the key objectives of this work were to (a) evaluate and analyze the impact of the three biochar variants on soil properties (quality and fertility indicators) and crop yield and (b) investigate the influence of mixing biochar with soil on the water extractable fractions (WEFs) from the resulting growth substrate. The investigation was done in the early phase (the first vegetation season) after the biochar amendment to the soil.

Materials and methods

Biochar preparation and characterization

There were three biochar types used in this study: walnut shell, wood chip, and plant waste biochars. Refuse WCB was purchased as a commercial product from the manufacturer Energo Zlatá Olešnice (Czech Republic). WCB was produced from a mixed softwood feedstock and registered by the Czech Ministry of Agriculture as an approved fertilizer (Registration No. 4867). PWB was another commercial product purchased from Sonnenerde GmbH (Riedlingsdorf, Austria), used previously for several studies (e.g., Brtnicky et al., 2023). It was manufactured through the pyrolysis of agricultural residues, such as cereal husks, sunflower peels, and fruit processing waste at 600 °C (batch ID: ba-at-34-2-3/ba000018). The properties of commercial biochars WCB and PWB were partially adopted from the manufacturers' data, while some properties (e.g., pH) were measured in the laboratory. Biochar from walnut shells was produced in-house as follows: The walnut shells (*Juglans regia* L.) were collected from Rielingshausen (Marbach, Germany), and were carbonized through autothermal pyrolysis in an industrial reactor (Biomacon GmbH, Rehburg, Germany), with the HTT in the reactor reaching approximately 700 °C. Except for one study (Mukome et al., 2013), the literature has seldom reported on the structure of the WSB carbon matrix before soil tests. A detailed physicochemical characterization of this in-house-produced biochar was undertaken. For this, WSB was oven-dried at 105 °C for 24 h and then shredded at 8,000 rpm to 0.2 mm in a ZM 200 centrifugal mill (Retsch GmbH, Haan, Germany). The methods for the detailed physicochemical characterization of WSB were based on our previous investigations (Nair et al., 2023a) and have been summarized in Supplementary Appendix S1.1 (Nair et al., 2023b).

Pot experimental setup

A 100-day pot experiment was conducted using tomatoes (*S. lycopersicum* L.) to assess and evaluate the impact of three different types of biochars on soil properties and tomato fruit yield. The experiment used potting soil derived from a silty clay loam (USDA Textural Triangle) *Haplic Luvisol* (WRB soil classification), collected from a farm near Troubsko village in the Czech Republic (49°10′28″N 16°29′32″E). The initial soil characteristics were as follows: total C 14.0 g kg^{-1}, total N 1.60 g kg^{-1}, P 0.10 g kg^{-1}, S 0.15 g kg^{-1}, Ca 3.26 g kg^{-1}, Mg 0.24 g kg^{-1}, K 0.23 g kg^{-1}, pH 7.3. The final potting soil was prepared by mixing the *Haplic Luvisol* (sieved to ≤2 mm) with fine quartz sand (0.1–1.0 mm; ≥95% SiO_2), coconut fibers, and vermicompost in a volume ratio of 7:1:1:1.

To study the effects of biochar on the yield of tomato fruits and soil properties, each type of biochar (section 'Biochar preparation and characterization') was mixed thoroughly with 9 kg of potting soil and placed in 11-L pots, each containing one tomato seedling (*S. lycopersicum* L.). The seven experimental variants, listed in Table 1, were replicated five times each. The doses of biochars were chosen in line with other previous pot experiments of the authors, (e.g., Brtnicky et al., 2023). The soil amended with a specific biochar type was denoted by adding the suffix 's' to the biochar's name. For example, soil amended with WSB biochar was referred to as WSBs. Experimental pots were randomly placed in a greenhouse where

Table 1. The experimental variants of biochar-amended soils used in this study

Variant	Amended by	Biochar weight (g)	Potting soil weight (kg)	Biochar content (w/w)
NC	No addition	—	9	0%
WSB 1%	Walnut shell biochar	100	9	1.1%
WSB 3%	Walnut shell biochar	300	9	3.2%
WCB 1%	Wood chip biochar	100	9	1.1%
WCB 3%	Wood chip biochar	300	9	3.2%
PWB 1%	Plant waste biochar	100	9	1.1%
PWB 3%	Plant waste biochar	300	9	3.2%

semicontrolled conditions were maintained (temperature 18/22 °C night/day; relative air humidity 70%; photoperiod according to the interval from June to August). Soil moisture was maintained at approximately 60% of water holding capacity throughout the experimental period. At the end of the experiment, tomato fruits were harvested from each plant and pot. The fruits were weighed, and the total yield per pot/plant was calculated. The average fresh weight of one piece of tomato fruit per variant was also recorded.

Soil analyses

Following the removal of the entire tomato plant, including the roots, soil samples (three per pot, 150 g each) were taken. These samples were homogenized, sieved through a mesh to a size of ≤2 mm, and used for further analysis. Air-dried soil was used for pH determination (ISO_10390, 2005), while soil samples stored at 4 °C were used for the determination of dehydrogenase activity (DHA), basal respiration (BR), and substrate-induced respiration (SIR). Freeze-dried soil samples (at −40 °C, stored at −18 to −20 °C) were used for enzyme activity measurements (β-glucosidase = GLU, N-acetyl-β-D-glucosaminidase = NAG, phosphatase = Phos, arylsulfatase = ARS, urease = Ure) and WEF analyses.

The DHA determination was based on the 2,3,5-triphenyltetrazolium chloride method (Doi and Ranamukhaarachchi, 2009); the results were expressed in μg TPF g^{-1} h^{-1}. Enzyme activities (GLU, NAG, Phos, ARS, and Ure) were measured on freeze-dried samples spectrophotometrically according to ISO_20130 (2018). Nitrophenyl derivates of natural substrates were used for the measurement of GLU, Phos, ARS, and NAG (at an emission wavelength of 405 nm), and urea was served as a substrate for Ure (measured at a wavelength of 650 nm), with values expressed in μmol NH_3 g^{-1} min^{-1} (urease) and μmol (p-nitrophenol) PNP g^{-1} min^{-1}. Further, the samples stored at 4 °C were used for BR and SIR measurements. This analysis, using a MicroResp device according to the James Hutton Institute protocol, was based on the method established by Campbell et al. (2003), with a colorimetric indication of CO_2 emission, with values expressed in μg CO_2 g^{-1} h^{-1}. BR was determined in assay without any supplements, and SIRs were carried out using various substrates: D-glucose (Glc-IR), D-trehalose (Tre-IR), N-acetyl-β-D-glucosamine (NAG-IR), protocatechuic acid (Pro-SIR), D-mannose (Man-IR), L-alanine (Ala-IR), and L-arginine (Arg-IR). Using enzyme activities, nutrient acquisition ratios were calculated based on equations (1) and (2) presented in Cui et al. (2022):

$$\text{C acquisition ratio} = \frac{\ln(\text{DHA} + \text{GLU})}{\ln(\text{DHA} + \text{GLU} + \text{NAG} + \text{Ure})}, \quad (1)$$

$$\text{N acquisition ratio} = \frac{\ln(\text{NAG} + \text{Ure})}{\ln(\text{NAG} + \text{Ure} + \text{Phos})}. \quad (2)$$

The carbon and nitrogen acquisition ratios directly depend on the secretion rate of carbon-utilizing and nitrogen-utilizing enzymes, respectively. Additionally, microbial resource limitation was estimated through the computation of vector length and angle (equations (3) and (4) in accordance with the theory of enzymatic stoichiometry proposed by Moorhead et al. (2016). Microbial C limitation aggravates with increasing vector length. The vector angle of <45° indicates microbial N limitation, whereas the vector angle of >45° indicates microbial P limitation. Vector length and angles were computed using the formulae based on Moorhead et al. (2016); ARCTG2 refers to arc tangent:

$$\text{Vector length} = \sqrt{\left(\frac{\ln(\text{DHA} + \text{GLU})}{\ln(\text{Phos})}\right)^2 + \left(\frac{\ln(\text{DHA} + \text{GLU})}{\ln(\text{NAG} + \text{Ure})}\right)^2}, \quad (3)$$

$$\text{Vector angle (rad)} = \text{ARCTG2}\frac{\ln(\text{DHA} + \text{GLU})}{\ln(\text{Phos})}; \frac{\ln(\text{DHA} + \text{GLU})}{\ln(\text{NAG} + \text{Ure})}. \quad (4)$$

The values of induced respiration were used to calculate the microbial functional diversity (MFD) according to Iovieno, Scotti and Zaccardelli (2021) as a Shannon's index according to equation (5):

$$\text{MFD} = -\Sigma\, pi \ln pi, \quad (5)$$

where pi is the ratio of the activity on a particular substrate to the sum of activities on all substrates. The WEF from all the biochar–soil variants (including the control) was analyzed as explained in Supplementary Appendix S1.2. The details of the statistical analysis are also available in Supplementary Appendix S2.

Results and discussion

General properties of the biochar

The common physicochemical properties in the procured commercial biochar types that are used in pot trials are presented in Table 2. The chemical characterization (proximate, ultimate analyses, and elemental concentrations) and the properties of the WEF of the WSB that is produced in-house are detailed in Tables 3 and 4, respectively. The comparison of the properties of three biochar variants showed that they have a similar content of total nitrogen (N), with the lowest value found in WSB. While WSB and PWB had similar pH ($CaCl_2$) and hydrogen (H) content, WCB and PWB displayed similar available magnesium (Mg^{2+}) content. When comparing WSB and WCB, both showed higher carbon content (C), lower oxygen content (O), and a lower (N + O)/C ratio compared with PWB, potentially due to similar organo-polymeric composition (e.g., probably higher lignin content) of their feedstocks (Pirayesh, Khazaeian and Tabarsa, 2012), compared with that of PWB. WCB and WSB also had higher K^+ levels than PWB, with Ca^{2+} being lower in WCB compared with WSB and PWB. The EC, V_{DFT}, and S_{BET} were the highest in WCB, while H, N, O, H/C, (N + O)/C, Mg^{2+}, Ca^{2+}, and Na^+ were the highest in PWB. WSB exerted the highest ash, C, P, and Na^+ content.

A comparison of these results reveals an interesting fact: WSB has a significantly higher ash content compared with the others, but it was not coupled with a smaller proportion of the carbonaceous part. Despite WSB showing both high ash and high carbonaceous fractions, it did not seem to affect other properties significantly. For instance, the S_{BET} parameter of WSB is still relatively high compared with PWB, which has about half the ash content of WSB. Intriguingly, the high ash content of WSB did not impact the electrical conductivity (EC) of the leachate. In fact, WSB exhibited the lowest EC, despite having similar amounts of water-soluble ions (K^+ and Na^+) and H content, compared with the other biochars. This suggests that water-soluble metals may be immobilized within the carbonaceous structure of the biochar. Another noteworthy

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Table 2. General properties of procured (from commercial suppliers) waste-derived biochar types used in pot trials

Property*	Unit	Wood chips biochar	Plant waste biochar
pH	—	9.6	9.60
Ash	%	4.61	15.90
EC	µS m^{-1}	740	327
V_{DFT}	cm^3 g^{-1}	0.24	
S_{BET}	m^2 g^{-1}	442.0	197.4
C	%	90.30	74.00
H	%	0.60	2.40
N	%	1.20	1.32
O	%	3.29	6.80
H/C	Molar ratio	0.08	0.39
(N + O)/C	Molar ratio	0.04	0.08
S	mg g^{-1}	<0.01	0.09
P	mg g^{-1}	0.51	1.30
Na^+	mg g^{-1}	1.47	0.86
K^+	mg g^{-1}	5.67	4.60
Mg^{2+}	mg g^{-1}	1.71	1.80
Ca^{2+}	mg g^{-1}	7.94	16.00

*Content (mean values) of elements and compounds in dry matter and water-extractable fraction of biochars: pH = pH determined in 0.01 M $CaCl_2$; Ash = ash content determined by loss on ignition at 550°C; EC = electric conductivity; S_{BET} = pore surface area (determined by the Brunauer–Emmett–Teller method); V_{DFT} = pore volume (by the Brunauer–Emmett–Teller method); C, H, N, O, P, S = total elemental content; Na^+, K^+, Ca^2, Mg^{2+} = available mineral content.

difference is seen in the PWB sample, which has a high content of oxygen and nitrogen. This results in increased polarity of the biochar, possibly leading to lower stability and higher degradability. Although a higher proportion of O and H in the molecule might be expected to result in higher acidity of the leachate, this was not observed. The high H/C ratio and low C content suggest that, compared with other biochars (Jafri et al., 2018), PWB is less aromatic and less carbonized, explaining its lower S_{BET}.

Table 4. Properties of water extractable fraction from walnut shell biochar that is produced with the in-house pilot scale reactor

Properties	Values	Units
pH	9.62–9.68	
EC	0.19	dS m^{-1}
DOC	17.17	mg L^{-1}
$SUVA_{254}$	0.50	L mg^{-1} m^{-1}
E2/E3	1.40	
NO_3^-	0.38 ± 0.00	mg L^{-1}
NO_3^-–N	0.08 ± 0.00	
PO_4^{3-}	2.01 ± 0.06	
PO_4^{3-}–P	0.66 ± 0.02	
SO_4^{2-}	2.64 ± 0.09	
SO_4^{2-}–S	0.88 ± 0.03	
Na^+	0.57 ± 0.02	
NH_4^+	0.53 ± 0.02	
NH_4^+–N	0.42 ± 0.01	
K^+	44.67 ± 0.74	
Mg^{2+}	1.05 ± 0.05	
Ca^{2+}	1.91 ± 0.17	

Previous studies comparing the impact of input pyrolysis feedstock on the properties of various biochar types have shown contrasting results. For example, a study by Ilic et al. (2022) found higher calcium content in wood biochar compared with agrowaste (PWB) biochar, as well as relatively high EC in PWB. These findings were opposite to those observed in the current study. In a comparison between pine nut biochar and the WSB presented in this study, the former was found to have significantly lower ash content but higher S_{BET}. Additionally, it was observed to be richer in O and N but lower in C (Chen et al., 2016). Similarly, another study comparing macadamia nutshell biochar to wood biochar reported slightly higher total carbon content in the former (Trigo et al., 2016). The pH values of both biochar types studied

Table 3. Chemical characterization of the walnut shell biochar produced in-house

Ultimate and proximate characterization		Unit	Elemental concentrations		Unit
C	91.44 ± 0.04	dry wt.%	B	0.07 ± 0.02	mg g^{-1}
H	2.21 ± 0.02		Na	1.82 ± 0.43	
N	1.18 ± 0.01		Mg	1.27 ± 0.00	
O	3.74 ± 0.02		K	5.76 ± 0.78	
VM	60.29 ± 7.26		Ca	12.6 ± 0.10	
Ash	31.98 ± 9.60		P	1.66 ± 0.03	
FC	7.72 ± 2.32		S	0.6 ± 0.12	
			Fe	0.67 ± 0.02	
			Al	0.46 ± 0.02	
			Cr	0.07 ± 0.01	
			Ni	0.09 ± 0.00	

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by Trigo et al. (2016) were found to be equal and less alkaline than biochars presented in this study. The nitrogen content in both nutshell biochar types was also comparable and different from the results of our investigation (Trigo et al., 2016).

Detailed physicochemical characterization of WSB

WSB is alkaline and contains high concentrations of ash (Table 3) relative to WCB and PWB (Table 2). WSB type is similar to those used in studies by Griffin et al. (2017) and Sial et al. (2022). This is possible because, unlike wood chips, walnut shell composition does not vary significantly across geographical regions unless they differ significantly in the long-term light intensity during the development of the nut (Zhao et al., 2011). WSB is well carbonized (molar H/C = 0.29) and highly porous, with a pore surface area (S_{BET}) and volume (V_{DFT}) of 245.4 $m^2\ g^{-1}$ and 0.11 $cm^3\ g^{-1}$, respectively. WSB is hydrophobic and contains high concentrations of exchangeable K+ (Table 4).

The PXRD diffractogram and Raman spectra of WSB are shown in Fig. 1a,b, respectively. The diffractograms show predominantly amorphous carbon with a broad peak around ~23° and weak reflections from graphite at planes [002] (2θ = 26.1°) and [100] (2θ = 42.3°). The sharp reflections at 20.7°, 26.5°, 50°, and 59.4° and the peaks between 67° and 68° indicate the presence of quartz. TEM examination (Fig. 2) shows various particle morphologies and sizes ranging from 100 nm to several micrometers. Typically, the platelet-like morphology displays the diffraction pattern of a hexagonal lattice (4.95 Å and approximately 120°), confirming the lattice constants of quartz in WSB as observed with PXRD. Diffraction peaks at 2θ of 30° and 36° may be from calcite. Such changes in biochar crystallinity are due to the incorporation of soil minerals and have also been reported (Sial et al., 2022). Interestingly, when the same walnut shells were used to prepare biochar on a lab scale, a minimal presence of such minerals was observed (Nair et al., 2023b), suggesting a potential (and unavoidable) contamination with sand during the industrial preparation (located in a farm) and transport of WSB. The carbon in WSB has an I_D/I_G of 0.64 ± 0.05 with an $FWHM_G$ of 66.2 cm^{-1}. The proportions of aryl and alkyl moieties, calculated from the ^{13}C-ssNMR spectra between the chemical shifts of 200 and 50 ppm (Supplementary Fig. S1), are 78.9% and 8.2%, respectively. Aryl C–O contributes about 11.5% of the total carbon.

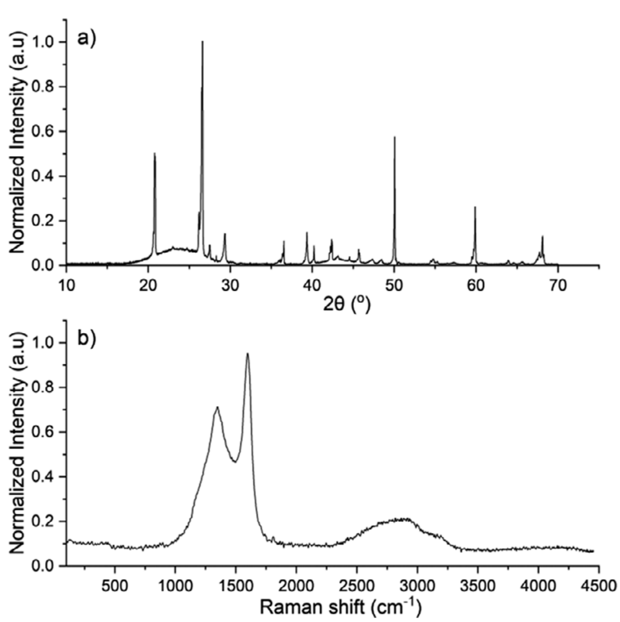

Figure 1. The diffraction peaks from PXRD (a) and the Raman spectra (b) of the walnut shell biochar.

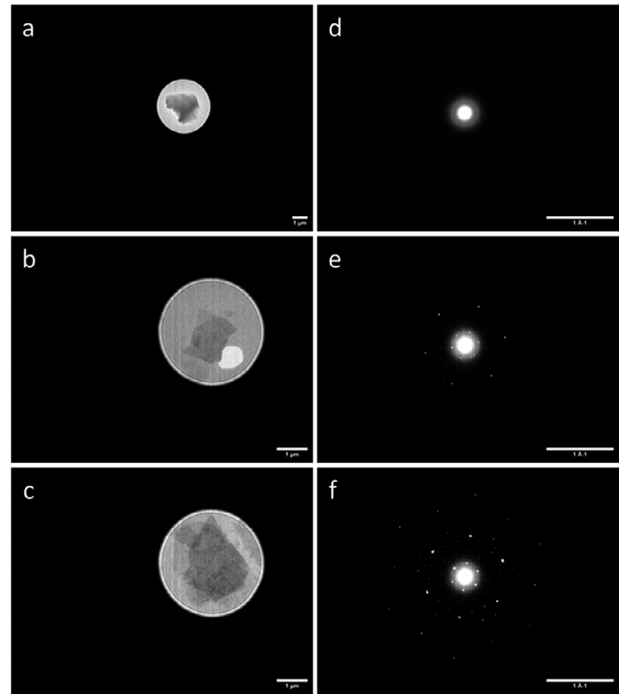

Figure 2. TEM Images of the region of interest (a–c) where electron diffraction images were acquired (d–f).

Effect of biochar types on soil pH

While only a minimal effect on soil pH was seen in PWBs, both WCB 3% and WSB 3% significantly increased soil pH. Moreover, WCB 1% caused a small increase in pH, while WSB 1% increased the pH value significantly (Fig. 3 and Supplementary Table S2). This contrasts with the findings of Kameyama, Iwata and Miyamoto (2017), who showed that agrowaste biochar had a more beneficial effect on neutralizing soil pH compared with WCB. Similarly, Zhang et al. (2019) reported a slight increase in pH with the amendment of agrowaste (maize straw) and hickory nut shell

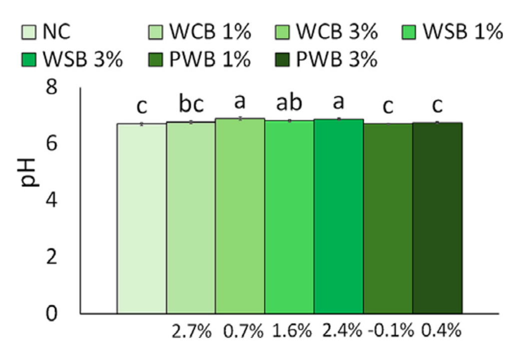

Figure 3. pH in soil amended with waste-produced biochar types and unamended control; mean values (n = 5) ± standard error of the mean (SE, error bars); the letters indicate differences (calculated by Tukey's HSD test) between variants at a statistical level of significance $p \leq 0.05$; numbers below columns show how much an average value of each variant differed from the value of control.

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biochar, whereas in this study, only a decreasing effect was found for nutshell biochar.

In the context of biochar properties, these observations are quite unexpected, considering that the pH of leachates from all biochars was very similar. When looking solely at solid biochar, PWB has the highest proportion of oxygen and hydrogen in its structure. This might lead one to expect that PWB contains a certain proportion of ionizing groups and, therefore, its pH might tend to be more acidic (Huff and Lee, 2015). However, PWB also has a high content of Ca^{2+} and a significant proportion of Mg^{2+}, which finally resulted in not much change to pH relative to the control. Accordingly, a similar effect can also be attributed to WCB and WSB, which also contain high levels of Ca^{2+} and Mg^{2+}. These biochars, however, have a lower proportion of oxygen- and nitrogen-containing groups, leading to a noticeable increase in pH. The soil pH had a negative relationship (principal component analysis [PCA]; Supplementary Fig. S2) with all enzymes and DOC (total organic carbon in the soil water extract), while it correlated positively ($p \le 0.001$) with the EC of water extract from the soil. It also correlated negatively with enzymes ARS and Phos (arylsulfatase and phosphatase; $p \le 0.001$, Pearson's correlation; Supplementary Fig. S3). The negative relationship of pH with DOC values in soil WEF agreed with the presumption of higher extractability of soluble soil carbon under lower pH and was in line with other reports (Zhang et al., 2020). Together with the negatively related pH and nutrient-transforming activity of soil enzymes, it aligns with the reported effects of biochar on both EC and nutrient-holding capacity in biochar-treated soil (Vijayaraghavan, 2020).

Effect of biochar types on water-extractable fractions of soil

As depicted in Fig. 4, the EC of soil water-extractable fraction (WEF) significantly increased with higher amendment doses of WCB and PWB (i.e., WCB 3% and PWB 3%), as well as with both doses (1% and 3%) of WSB. A negative correlation was observed between the addition of biochar and the leachability of DOC from the soil (Fig. 4 and Supplementary Table S2). This relationship is particularly significant in the variants WCB at 1% and 3%. Although there was an increase in soil EC, it is not entirely clear how EC was influenced, as WCB had the highest leachate EC and WSB had the lowest (Table 2). If the extracts themselves contributed to the soil EC, then WCB would affect it at both doses, and WSB only at the high dose. However, the results in Fig. 4 showed the exact opposite. In fact, increased soil conductivity can be caused by various factors, such as the release of ions and organic acids during the decomposition of organic matter or by chelating agents present in organic matter that can form complexes with metal ions, thereby contributing to higher EC. Another factor that increases EC in the soil is enhanced microbial activity, as the metabolism of organic compounds releases various metabolic products, including ions, into the soil solution (Chantigny, 2003; Kalbitz et al., 2003). However, as discussed in further paragraphs, microbial activity was not enhanced by biochar amendments. In order to evaluate if EC determination was not markedly influenced by a possible unexpected shift in the pH of WEF, the pH of water extracts was measured, and found that the values (Supplementary Table S1) corresponded to the results obtained from the pH measurement of soil suspension (Fig. 3).

As discussed earlier, biochar played a role in stabilizing soil nutrients, which could be associated with the higher EC as seen elsewhere (Vijayaraghavan, 2020). However, there was a trend of higher leachability of DOC from PWBs and WSBs compared with WCBs, consistent with findings from similar studies (Wei et al., 2019). PCA revealed a positive correlation between EC and E_2/E_3, as well as an inverse relationship with Ure. On the other hand, DOC was found to have a positive relationship with all enzymes, particularly with ARS and GLU (β-glucosidase; Supplementary Fig. S2). Correlation analyses indicated a significant ($p \le 0.001$) positive correlation between DOC and NAG ($p \le 0.001$), as well as DHA ($p \le 0.01$), and a negative correlation ($p \le 0.001$) between DOC and E_2/E_3 (Supplementary Fig. S3).

The specific UV absorption measured at a wavelength of 254 nm ($SUVA_{254}$) serves as an indicator of the carbon aromaticity in soil WEF (Fig. 5 and Supplementary Table S2). The aromatic content in DOC (indicated by $SUVA_{254}$) of the soil WEF decreased with biochar amendments, particularly with the 3% amendment doses of WSB and PWB. This is probably due to the lower DOC in biochar-amended soil compared to the control (Fig. 4 and Supplementary Table S2). These results are consistent with another study (Gao et al., 2020), where a lower $SUVA_{254}$ was reported for WEF from biochar-amended soil. The decrease in $SUVA_{254}$ is usually attributed to the adsorption of heavier aromatic DOC in the microporous structure of hydrophobic biochar, leading to a decrease in the E2/E3 ratio in the leached DOC, as shown in Fig. 5b. The control (NC) exhibited the lowest E_2/E_3 among all variants, especially PWB 3%. PCA revealed a negative correlation of $SUVA_{254}$ with BR, all IRs (except Glc-IR, Man-IR), and WF_average. E_2/E_3 was positively correlated with Glc-IR and negatively with Ure (Supplementary Fig. S2). Pearson's analysis revealed a weak negative correlation of $SUVA_{254}$ with Tre-IR, NAG-IR, WF_average (weak, $p \le 0.01$), and a moderate negative correlation with E_2/E_3 ($p \le 0.001$; Supplementary Fig. S3). Similar negative correlations of

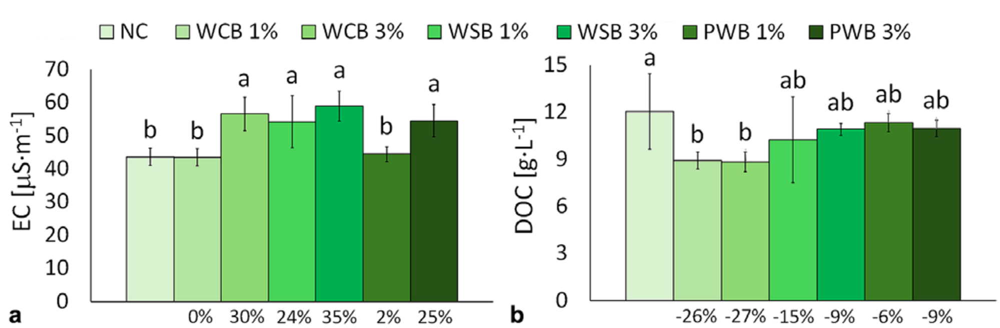

Figure 4. Electric conductivity (EC) (a) and total organic carbon (DOC) (b) in the water extractable fraction from the soil of all experimental variants; mean values ($n = 5$) ± standard error of the mean (SE, error bars); the letters indicate differences (calculated by Tukey's HSD test) between variants at a statistical level of significance $p \le 0.05$; numbers below columns show how much an average value of each variant differed from the value of control.

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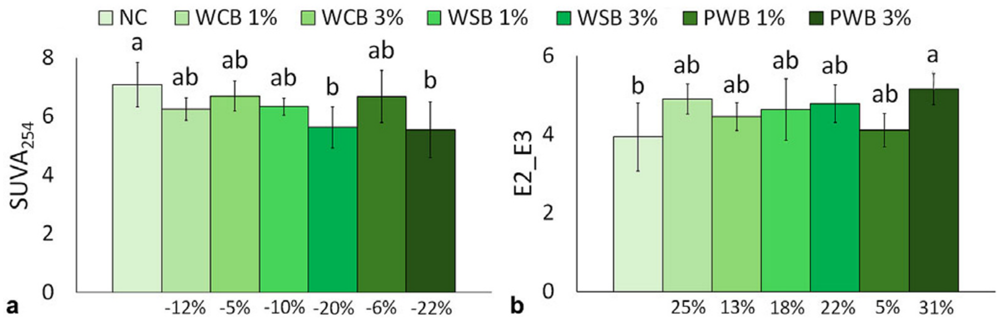

Figure 5. $SUVA_{254}$ (a) and the E_2/E_3 ratio (b) in the water extractable fraction from the soil of all experimental variants; mean values ($n = 5$) ± standard error of the mean (SE, error bars); the letters indicate differences (calculated by Tukey's HSD test) between variants at a statistical level of significance $p \leq 0.05$; numbers below columns show how much an average value of each variant differed from the value of control.

$SUVA_{254}$ with microbial indicators in soil have been reported in the literature (Azeem et al., 2023) and are attributed to the difficulty in the biotic breakdown of DOC.

Effect of biochar types on soil enzyme activity

Fig. 6 illustrates the microbial enzyme activity in the control and various types of biochar-amended soils. DHA, an enzyme crucial for SOM transformation, facilitates the breakdown of organic matter by participating in redox reactions. DHA helps the conversion of complex organic materials into simpler compounds (Alef and Nannipieri, 1995; Kaur and Kaur, 2021), which are then more accessible to plants and other microorganisms in the soil. In this work, DHA was found to be negatively impacted by the amendment of 3% WCB and 1% WCB, and both 3% doses of WSB and PWB tended to decrease its activity. Thus, DHA was affected the most negatively by WCB, the most porous biochar variant, while the impact of WSB was minimal. The decrease in DHA was found to be

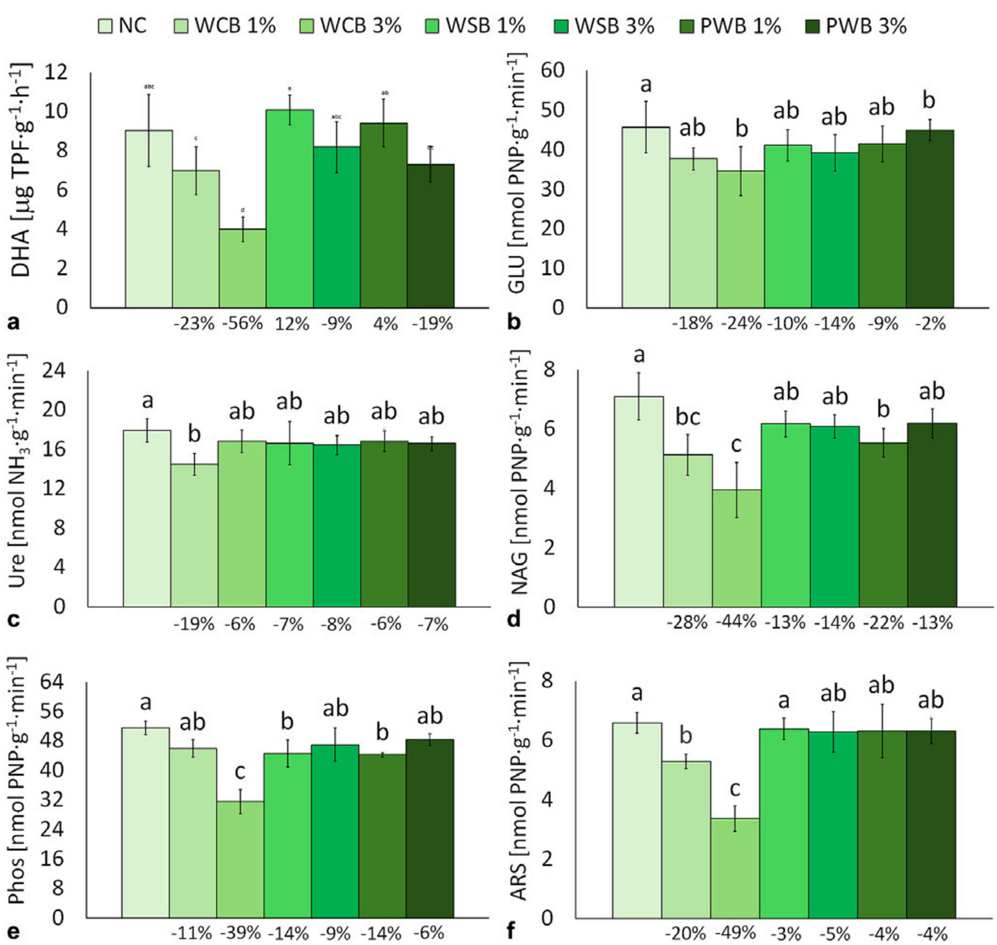

Figure 6. Enzyme activities in soil amended with waste-produced biochar types and unamended control; mean values ($n = 5$) ± standard error of the mean (SE, error bars); of dehydrogenase (DHA; a), b-glucosidase (GLU, b), urease (Ure, c), N-acetyl-b-D-glucosaminidase (NAG, d),phosphatase (Phos, e), arylsulfatase (ARS, f); the letters indicate differences (calculated by Tukey's HSD test) between variants at a statistical level of significance $p \leq 0.05$; numbers below columns show how much an average value of each variant differed from the value of control.

inversely proportional to biochar dosage, which is in agreement with previous studies on wood-based biochar (Demisie, Liu and Zhang, 2014). Higher doses of biochar (5% w/w) have been reported to decrease soil DHA (Brtnicky et al., 2019).

The observed decrease of DHA activity after the addition of biochars to soil can be attributed to several factors, such as biochars' porous structure and a large surface area, which can adsorb both enzymes and organic substrates, thereby decreasing their availability for biochemical reactions in the soil solution (Atkinson, Fitzgerald and Hipps, 2010). In addition, biochar can alter the microbial community structure in the soil by creating habitats that favor certain microbial groups over others, potentially reducing the population of microbes that produce DHA (Zimmerman, Gao and Ahn, 2011). This is also related to the creation of barriers to microbial movement and access to organic matter, which can reduce the interaction between microbes and SOM, thereby decreasing the overall enzymatic breakdown of organic materials (Biederman and Harpole, 2013). Furthermore, biochar can influence the redox potential of the soil due to its effects on soil aeration and moisture retention. These changes can indirectly affect the redox-sensitive processes in which DHA enzymes are involved (Cayuela et al., 2014).

PCA revealed a positive correlation between DHA and all enzymes except Urease (Ure), as shown in Supplementary Fig. S2. Pearson's analysis indicated a weak-to-moderate positive correlation ($p \leq 0.01$) between DHA and all other enzymes, except Ure, as shown in Supplementary Fig. S3. Other key enzymes, such as carbon-mineralizing β-glucosidase (GLU) and nitrogen-mineralizing urease (Ure), were minimally impacted (decreased) by biochar amendment, except in the case of WCB. GLU showed the most significant decline with the addition of 3% WCB, while Ure decreased with the addition of 1% WCB (Fig. 4b,c and Supplementary Table S2). Both 1% and 3% WCB exhibited a dose-dependent trend in Ure compared to the control, consistent with previous studies on wood biochar (Demisie, Liu and Zhang, 2014). Previous studies have shown that soil urease activity is generally positively influenced by the addition of wood biochar (Demisie, Liu and Zhang, 2014) and peanut shell biochar (Yao et al., 2021), contradicting our findings.

A positive-to-moderate ($p \leq 0.001$) correlation was observed between GLU and Phos, NAG, and ARS (Supplementary Fig. S3). N-acetyl-β-D-glucosaminidase (NAG) and arylsulfatase (ARS), enzymes involved in both nitrogen-carbon and sulfur mineralization, were both significantly decreased by WCB, with the lowest values observed at 3% WCB, while WSB had minimal effects on their activities (Fig. 6d,f and Supplementary Table S2). Pearson's analysis indicated a moderate positive correlation ($p \leq 0.001$) between NAG and ARS (Supplementary Fig. S3). Phosphatase (Phos) was the only enzyme that was negatively influenced by all of the biochar variants, with WCB 3% causing the lowest Phos value compared to the control (Fig. 6e and Supplementary Table S2). Phos showed a positive correlation (moderately to highly, $p \leq 0.001$) with GLU, NAG, and ARS (Pearson's analysis; Supplementary Fig. S3). It was observed that WSB was not as effective in stimulating soil phosphatase activity as peanut shell biochar, as reported by Yao et al. (2021). The measured enzyme activities helped to establish a balance between the processes of soil nutrient acquisition by microorganisms and plants, as indicated by the carbon and nitrogen acquisition ratios and by indicators of C limitation ('Vector') and N/P limitation ('Angle') (Table 5). These results indicated that carbon acquisition (indicated by the C acquisition ratio) was increased by PWB 3% and decreased by WSB 3% and WCB 3% compared with the control. Due to biochar addition, the soil C limitation was highest for PWB 3% and lowest for WCB 3% compared to the control. The N acquisition ratio was most increased by WCB 3% and most decreased by WCB 1% (Table 5). The 'Angle' parameter, an indicator of nitrogen and phosphorus limitation, shows that WCB 3% tends toward N limitation, and the variants WCB 1% and PWB 3% hinge toward P limitation.

In summary, WSB did not change the soil C and N content or the demand for nutrients by soil microbes compared to the unamended control soil, in contrast to the positive effects reported for some nutshell biochar types (e.g., peanut shell biochar) on soil N and P content (Yao et al., 2021). The only significant effect of WSB was a presumed negative priming effect on carbon stabilization, indicated by a lower C acquisition ratio (and reduced soil carbon limitation) compared with the control at dose 3%.

Effect of biochar types on soil respiration and microbial functional diversity

BR is a key indicator of aerobic catabolic activity in soil. Higher rates of BR can indicate a more active microbial community, which is generally associated with better soil fertility and structure. Conversely, lower respiration rates might suggest less microbial activity, which could be due to factors such as nutrient deficiency, pollution, or heavy metal contamination. As shown in Fig. 7a, BR was significantly increased by 3% PWB, whereas the other variants led to BR values that were comparable to the control (Fig. 7a and Supplementary Table S2). Glucose-induced soil respiration (Glc-IR) was increased at higher doses, that is WCB 3% and PWB 3%, and at WSB 1%. On the contrary,

Table 5. Enzyme activities and nutrient acquisition ratio in soil amended with waste-produced biochar types and unamended control

Variant	C acquisition ratio	N acquisition ratio	C, N, P limitation		
			Vector	Angle	° (ang. degrees)
NC	0.913 ± 0.01 b	0.742 ± 0.01 bc	1.600 ± 0.02 c	0.886 ± 0.01 bc	50.77 ± 0.64 bc
WCB 1%	0.913 ± 0.00 b	0.713 ± 0.02 d	1.625 ± 0.02 b	0.909 ± 0.01 a	52.08 ± 0.75 a
WCB 3%	0.895 ± 0.01 d	0.766 ± 0.01 a	1.599 ± 0.03 c	0.850 ± 0.002 d	48.69 ± 0.13 d
WSB 1%	0.913 ± 0.00 b	0.737 ± 0.04 bc	1.620 ± 0.01 b	0.878 ± 0.01 c	50.30 ± 0.73 c
WSB 3%	0.908 ± 0.00 c	0.747 ± 0.01 bc	1.612 ± 0.03 c	0.887 ± 0.04 bc	50.79 ± 2.26 bc
PWB 1%	0.911 ± 0.00 bc	0.732 ± 0.02 c	1.619 ± 0.01 b	0.879 ± 0.01 c	50.38 ± 0.40 c
PWB 3%	0.919 ± 0.00 a	0.742 ± 0.01 bc	1.640 ± 0.02 a	0.893 ± 0.01 b	51.15 ± 0.71 b

Note: Mean values ($n = 5$) ± standard error of the mean (SE, error bars); the letters indicate differences (calculated by Tukey's HSD test) between variants at a statistical level of significance $p \leq 0.05$.

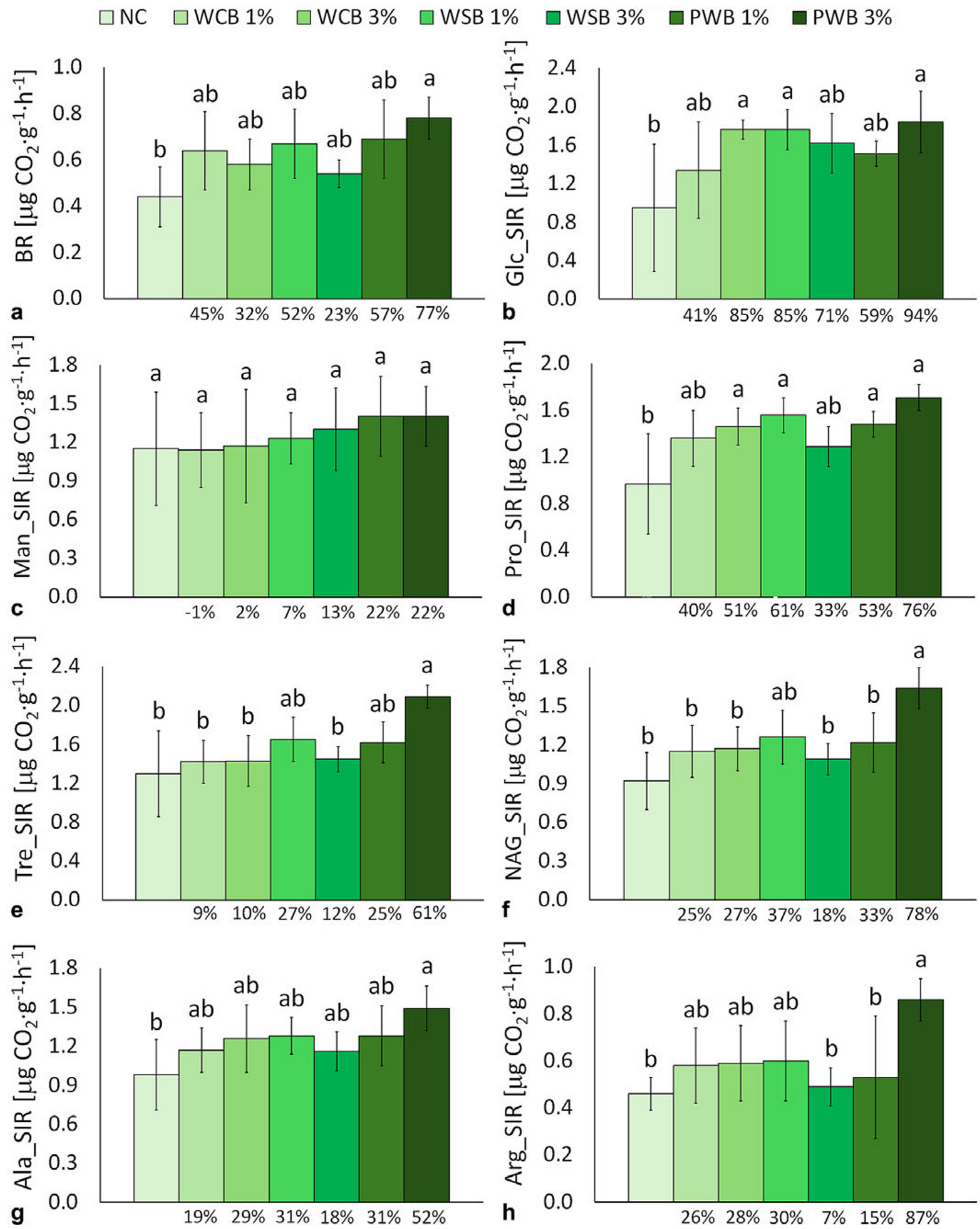

Figure 7. Respiration (basal and substrate-induced) in soil amended with waste-produced biochar types; mean values ($n = 5$) ± standard error of the mean (SE, error bars); of BR (a), SIRs induced by D-glucose (b), D-mannose (c), protocatechuic acid (d), D-trehalose (e), Nacetyl-b-D-glucosamine (f), L-alanine (g), L-arginine (h); the letters at the bottom of bars indicate difference (calculated by Tukey's HSD test) between variants at a statistical level of significance $p \leq 0.05$; numbers below columns show how much an average value of each variant differed from the value of control.

D-mannose-induced respiration (Man-IR) was indifferent among all biochar types and doses (Fig. 7b,c and Supplementary Table S2). Soil respiration induced by protocatechuic acid (Pro-SIR) was increased by WCB 3%, PWB 3%, and WSB 1% (Fig. 7d and Supplementary Table S2). Respiration induced by D-trehalose and N-acetyl-β-D-glucosamine (= monosaccharides, Tre-SIR, NAG-SIR) and by amino acids L-alanine (Ala-IR) and L-arginine (Arg-IR) were all increased by PWB 3% (Fig. 7e–h and Supplementary Table S2). Similarly, macadamia nutshell biochar has also been showcased to contribute to the stimulation of soil respiration (Xu et al., 2018).

The calculation of MFD values, as reported in Table 6, showed a tendency to higher abundances of various microbial groups coupled with all amendments. However, only the amendment of 3% PWB increased MFD significantly in comparison to the control. This may lead to improved microbial ability to use variable carbon sources. In contrast, the soil amended with 1% nutshell biochar (a mix of peanuts, almonds, and walnuts) was reported as beneficial for microbial community performance in crop yield stimulation and for microbial abundance in soil (Aziz et al., 2020). Additionally, Xu et al. (2018) highlighted the benefits of WSB on both BR and the microbial community. These observations are more in line with the results of Glc-IR and Pro-IR determination in the WSB 1% variant, which showed increased values as the only variant amended by a 1% dose. PCA showed that the most determined IR types (except Glc-IR and Man-IR), and BR were positively related to each other (Supplementary Fig. S2). Pearson's correlation showed a significant

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Table 6. Microbial functional diversity in soil amended with the different biochar variants

Variant	MFD
NC	1.887 ± 0.030 b
WCB 1%	1.904 ± 0.020 ab
WCB 3%	1.896 ± 0.020 ab
WSB 1%	1.897 ± 0.020 ab
WSB 3%	1.895 ± 0.020 ab
PWB 1%	1.891 ± 0.030 ab
PWB 3%	1.910 ± 0.010 a

Note: Mean values (n = 5) ± standard error of the mean (SE, error bars).

($p \leq 0.05$) positive relation among all IRs except BR, Glc-IR, and Man-IR (Supplementary Fig. S3).

Effect of biochar types on the yield of tomato fruits

The average yield of tomato fruit (WF_average in grams) per variant was comparable across different types of biochars and the control (Fig. 8a and Supplementary Table S2). PCB 3% and WSB 3% show a similar tendency to a higher yield of 50 and 48 g, respectively, in WF_average when compared with the yield (28 g) of the control. PCB 1% shows a lower yield of 42 g compared to WSB 1% of 46 g. Nevertheless, the differences were not statistically significant. The total harvested tomatoes (expressed as yield per plant in grams, WF_sum) per each experimental variant were lowest for WSB 3% at 200 g (comparable to the control at 210 g) and highest for PWB 1% at 320 g on average (Fig. 8b and Supplementary Table S2). However, the PWB 1% variant was the only one that exerted a statistically significant increase in fruit yield compared to the control.

Since WSB is hydrophobic, it might require additional aging time in the soil before it significantly affects the soil–plant–microbial chemical system. According to Safaei Khorram et al. (2020), the impact delay from the WSB application was reduced to 1 year when the biochar was added 4 months before planting. This delay might explain the nonsignificant effect of WSB on microbial BR, enzyme activity, and tomato yield in this 100-day study, in which WF_sum of WSB 3% was lower than that of WSB 1% and even of the control without any biochar. The carbon matrix degradability may affect such time delay. In WSB, calcium appears to be predominantly bound as inorganic carbonates. Additionally, the soil pH was already within the nutrient solubility range before the biochar amendment. Therefore, any potential nutrient availability from WSB is likely due to the direct addition of exchangeable cations rather than any nutrient retention mechanism, as observed by Griffin et al. (2017).

The WSB carbon matrix is predominantly aromatic and unordered with a high defect density. Such high aromatic fractions in biochar protect the SOM through the formation of soil macroaggregates, reducing the accessibility of C pools to microbes (Wang et al., 2017). This increased recalcitrance of biochar carbon and the protection of SOM could account for the observed reduction in the mean value of respiration (BR and SIR) when the WSB loading rate was increased from 1% to 3%. This reduction in microbial biomass was also reported (Sial et al., 2022) for WSB (at an HTT of 600 °C) during a similar 120-day incubation test. However, in contrast to our results, Sial et al. also reported an increase in urease and phosphatase activities and a reduction in glucosidase activity for the same treatment. Furthermore, one question that could not be answered based on these results is why WCB did not cause a similar reduction in microbial respiration despite being more aromatized than WSB. This necessitates further testing for confirmation and clarification. A similarly microporous, but low-ash, WSB enhanced the growth of bamboo plants in a 160-day pot experiment by Wang et al. (2019). However, this was primarily due to the remediation effects of biochar, namely, the immobilization of heavy metals and the 'repairing' effect of biochar on the growth medium, which was heavily contaminated with heavy metals. PCA revealed a positive relationship between WF_average and WF_sum with most induced respirations (Supplementary Fig. S2). Although previous studies (Aziz et al., 2020) reported beneficial effects of nutshell biochar types on crop yield, the initial hypothesis that WSB may positively affect tomato yield could not be confirmed with any statistical significance based on the observed results.

Conclusion

The influence of biochar derived from lignin-rich feedstocks on soil was comprehensively investigated using the WSB produced in-house and commercially available WCB, and these results were compared with those of biochar from plant wastes (PWB), another commercial product. This investigation included a 100-day pot experiment with tomatoes and a detailed physicochemical characterization of the carbon matrix in WSB. The PWB had a weak or no positive effect on soil enzymes, similar to WSB. Among all the tested biochar variants, only PWB 1% has increased tomato yield (measured by the weight of all harvested tomatoes per plant in the pot variant) compared to the control. PWB also increased C mineralization

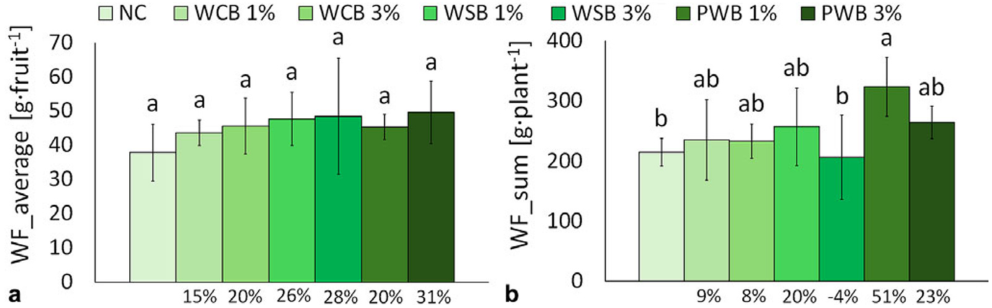

Figure 8. Yield of tomatoes fruits harvested from plants grown in soil amended with waste-produced biochar types; mean values (n = 5) ± standard error of the mean (SE, error bars); WF average (a) = mean weight of one tomato per each variant (in grams), WF sum (b) = weight of all harvested tomatoes per a pot and variant (in grams); letters at the bottom of bars indicate difference (calculated by Tukey's HSD test) between variants at a statistical level of significance $p \leq 0.05$.

measured as BR and SIR due to the higher degradability of this biochar with relatively lower C content and higher H/C ratio. On the contrary, detailed WSB characterization revealed hydrophobicity, high carbonization, and aromaticity of organic carbon structured in a way that decreases degradability by soil microorganisms. WSB did not decrease soil enzyme activity, similar to WCB, which showed similar C, N, O, and K^+ content and a high specific surface compared to WSB. Nevertheless, the addition of WSB (3% w/w) slightly increased soil carbon stabilization, as indicated by the C acquisition ratio and the C limitation value. Unlike PWB, WSB did not improve the yield of tomato fruits as compared to the unamended control soil. However, WSB also did not cause any negative priming effect on soil microbial mineralizing activity as WCB did.

Given the high recalcitrance of WSB and depending on the initial soil quality, it likely requires more time to age in the soil to manifest any measurable short- and/or long-term benefits to crop yield and soil characteristics. Future studies with LRFs and other biochar variants are recommended with longer aging durations and also tests against co-application with other types of organic amendments containing less stabilized organic nutrient sources, such as compost. Furthermore, the authors believe that it is paramount that future investigations on the soil application of biochar derived from LRF are not restrained by the positive results bias. Not all such biochar may have the intended outcomes during soil amelioration, especially during the (field/pot) testing duration. They must be reported, critically analyzed, and compared with other biochar variants to fully understand the complex interactions in the biochar–soil–microbe ecosystem.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S1742170525100082.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contribution. Conceptualization: J.H., D.W.; Drafting: R.R.N., J.H., T.B.; Experiments: R.R.N., T.N., T.H., Y.K.; Formal analysis: R.R.N., J.H., J.K., T.N.; Funding acquisition: D.W., M.B.; Methodology: R.R.N., T.H., Y.K., A.K., T.B.; Resources: D.W., J.H., M.B.; Supervision and project administration: D.W., M.B.; Writing – review and editing: J.H., R.R.N., J.K., D.W., M.B.

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Competing interests. The authors declare that they have no known competing financial interests and/or personal relationships with other people or organizations that could have appeared to influence the work and/or the reported results and discussions.

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