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Impact of modified biochar on phosphorus fractionation and biochemical properties for different soils

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Abstract

Modified (metal oxide) biochar is widely used for the remediation of degraded soils, but there has been limited research work on its effect on phosphorus fractionation and biochemical properties under different soil conditions. Therefore, this study examined the effects of a nonmodified wheat straw biochar (WBC) and a magnesium-modified wheat straw biochar (Mg-WBC) on phosphorus fractions, soil chemical properties, enzyme activity and microbial biomass in Qiyang (QY) and Harbin (HAR) soils. The study included a control, two WBC doses (1 and 2.5% w/w), and two Mg-WBC levels (1 and 2.5% w/w). The results revealed that WBC and Mg-WBC significantly improved soil characteristics. Alkaline phosphatase and catalase activities were significantly enhanced, while acidic phosphatase after an increase at 1% dose then decreased at 2.5% dose with both biochars. Both microbial biomass carbon and phosphorus showed significant increases when QY and HAR soil received 2.5% dose of biochar. Labile-P significantly increased by 28–77% and 15–47% in QY and HAR soil, respectively, for each of the two levels of WBC and Mg-WBC amendments. The concentrations of moderately-labile-P decreased (2–3% and 3–6%, in QY and HAR soil, respectively) at 1% rate, while increased (9–11%, and 3–6%, in QY and HAR soil, respectively) at the 2.5% rate of both types of biochar. However, the nonlabile-P decreased by 27–38% and 15–35% in QY and HAR soil, respectively. Mantel's test showed a stronger effect for soil organic carbon in the QY soil, while available phosphorus and microbial biomass carbon were more affected in the HAR soil. The partial least square path model (PLS-PM) analysis showed a better effect of biochar on acidic soil, and Mg-WBC significantly improved P availability in both soils. Our findings suggest that Mg-modified biochar would be an appropriate strategy for improving soil fertility, P-availability in labile and moderately labile forms, with specific recommendations for their use in different soil conditions.

Keywords Acidic soils, Enzymatic activities, Microbial biomass, Modified biochar, P fractions

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Introduction

Phosphorus (P) is a very important macro nutrient element, vital for crop growth and biochemical processes [1]. An appropriate level of P availability can improve soil productivity [2]. However, the improper use of P-containing fertilizers may cause severe P loss due to leaching, surface runoff, and soil erosion. It has been estimated that total P content in soils would be approximately 570 mg kg^{-1}, while only 0.1% of this amount would be available for plants [2]. The remaining P would be mainly adsorbed on soil colloids or complexed with Ca, Fe and Al [1]. Therefore, acquiring further knowledge on aspects related to sequential P fractionation, under different conditions (including the effects due to amendments), would be crucial to increase the understanding of variations in P availability for soils.

The availability of P to plants depends on different P fractions, which exist in both organic (Po) and inorganic (Pi) forms, with a very small amount of Pi being available [1]. To measure soil P fractions, Hedley et al. [3] proposed a standard protocol of P fractionation, based on the availability of P to microbes and plants. Thus, soil P fractions can be divided into labile P (resin-P or H_2O-P, $NaHCO_3$-Pi, and $NaHCO_3$-Po), moderately labile-P (NaOH-Pi and NaOH-Po, Dil-HCl-P) and stable or nonlabile P (Con-HCl-P and residual-P) [3]. Labile-P includes bioavailable P for plants and different microbes, whereas moderately-labile-P that is bound to Fe and Al, under anoxic conditions may be reduced and could become available for plants [4]. The nonlabile P fractions are adsorbed on to soil colloids or may be involved in the formation of complexes with other elements and could be released through mineralization, or when soil pH reaches enough low values [5]. Soil pH and organic matter are among the most important factors that can increase or decrease P availability in the soil solution. It must be stressed that part of the nonlabile P that is adsorbed onto Fe and Al or soil clay particles may become available due to the activity of phosphatase enzymes [6]. In addition, soil amendment can also increase P availability by improving the status of soil organic matter, pH and fertility [2]. As specific example, Sui et al. [1] applied biochar to a black soil, reporting an increase in the labile P fractions and a decrease in the residual P fraction, which suggests that certain amendments may promote the transformation of nonlabile P into labile P fractions in soils. Similarly, Wu et al. [7] observed that the addition of biochar to a red acidic soil resulted in 159–255% increase in bioavailable P fractions. Deng et al. [8] applied rice straw biochar into Ferralsols acidic soil, reported a tenfold increase in labile P as compared to no biochar treatment.

Biochar is prepared from heating of plant biomass (straw, twigs, trunk, and agricultural waste) and poultry and animal manure among them under the absence or limited oxygen supply [9]. Biochar is commonly characterized by its large surface area, porous structure, and diverse surface functional groups. It has a high carbon (C) content and is known for enhancing carbon sequestration, improving water holding capacity, boosting soil aeration and pH, and exhibiting a strong adsorption capacity [10]. These properties make biochar a good conditioner to enhance soil quality and to promote remediation [11], even when compared with some low-cost materials and by-products [12, 13].

Biochar addition to soil may cause a marked increase in the bioavailability of soil nutrients, particularly P [1]. Biochar produced at low temperature (400 °C) contains high rates of labile P but, as the pyrolysis temperature rises, the labile-P slowly decreases and is converted to more stable forms. Therefore, the pyrolysis temperature is a key aspect in regards to P availability [1]. Previously Phuong et al. [14] documented that pristine biochar reduced effectiveness in the sorption of anions (such as PO_4^- and NO_3^-), which would be due to the negatively charged surface of nonmodified biochar. Appropriate modifications to biochar should be recommended to enhance its potential for anion retention in soil ecosystems [6]. In this regard, biochar modifications using magnesium (Mg), calcium (Ca), and aluminum (Al), have been previously studied, where cations are precipitated on the surface of biochar during pyrolysis; thus, creating bridges to adsorb anions such as phosphate [15]. Specifically, $MgCl_2$ is widely used for the modification of biochar, as done by [16], that revealed a sixfold increase in P adsorption, and Li et al. [15] reporting a maximum P adsorption capacity of 398 mg g^{-1}. Furthermore, Peng et al. [17] studied the effect of modified biochar on P dynamic and soil health, and concluded that modified biochar has the potential to reduce P leaching and improve soil health. Additionally, it should be noted that most of the studies dealing with P removal by means of modified biochar have focused on wastewater, while there is a lack of studies regarding the effect of modified biochar on P fractions for different soils.

In view of background, the aims of this study were: (1) to elucidate the effect of nonmodified wheat straw biochar (WBC) and Mg-modified biochar (Mg-WBC) on soil P fractions for different soils and (2) to assess the effect of modified and nonmodified biochar on soil physicochemical and biological properties. The results of this investigation could be of great value to promote the appropriate recycling of agricultural wastes or by-products used to prepare biochar, as well as to improve the management of soils where biochar materials are applied, considering the availability of nutrients provided (with special focus on P) and the overall potential to ameliorate soil conditions and enhance ecosystems sustainability.

Materials and methods

Experimental site, soil sampling and biochar preparation

Two different sites were selected for soil sampling, located in Qiyang (26°45′N, 111°52′E, 120 m altitude), Hunan Province, and in Harbin (45°40′N, 126°35′E, 151 m altitude), Heilongjiang Province, China. The sites were named as Qiyang red soil (QY) and Harbin black soil (HAR). The mean annual temperature and precipitation at QY were 18.6 °C and 1431 mm, while in HAR there were 3.7 °C and 533 mm, respectively. The soil types and textural class in QY and HAR were Eutric Cambisol and Ferrosols (clay) [18] and Udic Mollisol (Silt loam) [19], respectively. The soil samples were randomly (five different points) collected from 0 to 20 cm surface layer at each site. After removing the visible stones, roots and other plant residues, the soil was mixed to make a composite sample, air dried, grounded, and sieved through 2 mm mesh and stored for further analyses.

Biochar was prepared from grounded (1 mm) wheat straw, which was provided by the Qiyang experimental station. After grounding, then a portion of the wheat straw was chemically modified according to Haddad et al. [20]. Briefly 200 g of wheat straw were doped in 2000 ml of 20% (w/w) $MgCl_2.6H_2O$ solution, then shaken for 6 h, filtered, and dried at 60 °C for 24 h. Afterward both the raw and $MgCl_2.6H_2O$-doped dried wheat straw materials were pyrolyzed (so, with no oxygen supply) for three hours at 350 °C. After pyrolysis, the resulting nonmodified and Mg-modified biochar materials were named as WBC and Mg-WBC, respectively. Further details can be found in the schematic diagram (Fig. 1).

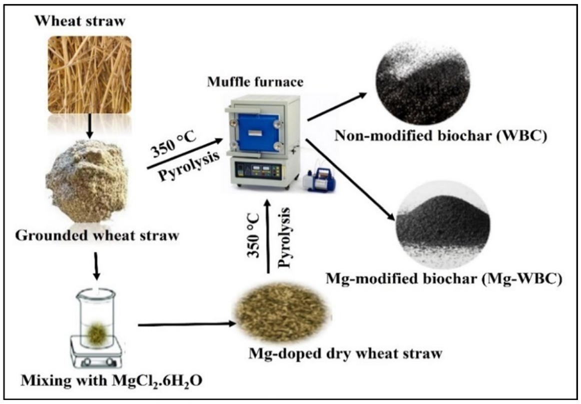

Fig. 1 Schematic diagram of pristine biochar preparation and modification

Amendments and experimental set-up

The research was carried out by means of a laboratory incubation experiment, which lasted for a total of 90 days. Five different treatments were applied, namely: control (CK) without amendments; two rates of nonmodified biochar (WBC): WBC_1 and $WBC_{2.5}$ (10 and 25 g biochar kg^{-1} soil) and two rates of Mg-modified biochar (Mg-WBC): Mg-WBC_1 and Mg-$WBC_{2.5}$ (10 and 25 g biochar kg^{-1} soil) which were arranged in a completely randomized block design with three replicates. The specific procedure consisted in manually mixing samples (200 g of soil), with their particular treatment materials (at the various required doses), putting the resulting mixtures into 1000 mL glass bottles, then moistened to 60% of the water field capacity with deionized water, as per Khan et al. [21]. Moreover, all the bottles were closed with a perforated plastic sheet to reduce excessive water evaporation; the weight of each bottle was recorded for a correct adjustment of soil moisture (weighing each bottle thrice a week and adding water if necessary). Then, the bottles were kept in the incubator at room temperature (25 ± 0.3 °C).

Soil and biochar analyses

For soil and biochar pH measurement, 1:2.5 and 1:20 of water suspensions were prepared, respectively, according to Inyang et al. [22], and read using a pH meter (Mettler Toledo 320-S, Switzerland). The total C and N in soil and in biochar were quantified using a C/N analyzer (Elementar, Vario Max, Germany) and the carbon and nitrogen (C: N) ratio was calculated by dividing the values of total C by total N. The morphology of biochar was determined through SEM (scanning electron microscopy) and its elemental composition was elucidated through EDS (energy-dispersive X-ray spectrometry). The percent yield of biochar was calculated dividing the amount of raw material by the prepared biochar, while the percent ash content was measured by combusting the biochar samples at 500 °C for 1 h in a muffle furnace [23]. Soil total P and total potassium (K) were quantified by means of the wet digestion method [24]. The sodium bicarbonate (0.5 M $NaHCO_3$ at pH 8.5) extraction method was used for the determination of available phosphorus (AP), whereas the ammonium acetate (0.5 M) method was employed for the measurement of available potassium (AK) [25–27]. Soil organic carbon (SOC) was determined by using the wet digestion method [28]. Microbial biomass carbon (MBC) was measured by extracting soil samples with 0.5 M K_2SO_4 following the chloroform fumigation method of Vance et al. [29]. Soil microbial biomass phosphorus (MBP) was determined according to the standard protocol of Brookes et al. [30], where the fumigated and nonfumigated soil samples were extracted with sodium bicarbonate ($NaHCO_3$ at 0.5 M). All the soil parameters were measured only at the end of the 90-d incubation. Table 1 shows the main physicochemical characteristics corresponding to the two soils and biochar materials used in the current study.

Determination of soil enzymatic activity

The activities of acidic phosphatase (AcP), alkaline phosphatase (AlP) and catalase (Cat) were measured according to the methods described by Tabatabai [31]. Briefly, modified universal buffer (MUB), at pH 6.5 and 11, were used for AcP and AlP enzymes, respectively, measuring the yellow color intensity at 410 nm, by means of a spectrophotometer (UV-2450 Shimadzu, Japan). The catalase activity was determined based on the recovery of hydrogen peroxide (H_2O_2). Briefly, 2 g of air-dried soil samples were treated with 0.3% H_2O_2, with the resulting filtrate being subsequently titrated with 0.1 mol L^{-1} $KMnO_4$ after 20 min of reaction.

Sequential phosphorus fractionation

The protocol of Hedley et al. [3] was used for phosphorus fractionation. The Hedley fractionation method differentiates between resin-P (res-P), $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pi, NaOH-Po, HCl-P (Dilute and Concentrated HCl), and residual-P (resd-P). Briefly, 0.5 g of soil (oven dry base) was first extracted with 30 ml distilled water and resin strips in a 50 ml centrifuge tube, shaken for 16 h. Then, the residual soil was sequentially extracted with 0.5 M $NaHCO_3$ (pH 8.5) ($NaHCO_3$-P), 0.1 M NaOH (NaOH-P), and 1 M HCl (Dil-HCl), followed by 16 h shaking (similar in all steps). After this step, 10 ml of concentrated HCl (Con-HCl-P) was added to the solution and heated for 10 min at 80 °C in a water bath, then added 5 ml of concentrated HCl and distilled water to raise the volume up to 50 ml. Finally, the residual soil was digested with H_2SO_4 and H_2O_2 to measure the residual-P. After each step, the supernatant was collected by centrifugation at 25,000 ×g for 10 min at 4 °C. The $NaHCO_3$, NaOH and Con-HCl extracts were divided into two parts: one part was directly analyzed for inorganic phosphorus (Pi) while the other part was digested with ammonium persulfate to determine total phosphorus (Pt). The organic-P (Po) was calculated by subtracting Pi from Pt (Pt–Pi = Po). The P concentration in each step was determined at 882 nm with a spectrometer according to Murphy and Riley [26].

Statistical analysis

Calculations corresponding to P fractions were initially performed using MS Excel, version 2016. To find the effect of WBC and Mg-WBC on biochemical properties of soil and P fractionation, analyses of variance (ANOVA) were performed, using SPSS 22 (IBM) followed by least significant difference (LSD) estimation. To meet the assumptions of ANOVA ($p < 0.05$), all the data were checked for normality and homogeneity of variances. All the graphs were plotted by using Origin 2019. R software (version 4.3.1) was used to find the relations between soil properties, soil microbial biomass, soil enzymatic activity and P fractions, based on Mantel's test and Spearman's rank correlation coefficient. Furthermore, the partial least square path model (PLS-PM) was used to find variables that had a greater impact on the P fractions. The observed variables can be explained by the latent variables, which in they were soil properties (SP), microbial biomass (MB), soil enzymes (SA) and P fractions (PF), this being analyzed by the R software (version 4.3.1).

Table 1 Main physicochemical characteristics of the soils (QY and HAR), pristine wheat straw biochar (WBC) and magnesium-modified wheat straw biochar (Mg-WBC) used in the research

	QY	HAR	WBC	Mg-WBC
pH (H_2O)	5.69 ± 0.05	6.75 ± 0.01	10.34 ± 0.02	9.68 ± 0.03
EC (H_2O) (mS cm^{-1})	–	–	6.01 ± 1.03	8.13 ± 4.20
Total organic carbon (%)	0.85 ± 0.08	0.98 ± 0.20	53.03 ± 3.12	48.76 ± 5.21
Total nitrogen (%)	0.11 ± 0.02	0.15 ± 0.11	1.23 ± 0.01	1.32 ± 0.02
Total phosphorus (g kg^{-1})	0.51 ± 0.01	0.55 ± 0.02	2.03 ± 0.02	2.53 ± 0.04
Total potassium (g kg^{-1})	13.61 ± 0.08	25.3 ± 0.20	0.59 ± 0.01	1.09 ± 0.01
Available P (mg kg^{-1})	12.40 ± 0.20	51 ± 0.30	–	–
Exchangeable K (mg kg^{-1})	91.63 ± 3.2	175 ± 5.60	–	–
C/N	7.71 ± 0.50	6.5 ± 0.30	43.11 ± 0.12	36.10 ± 2.30
Ash (%)	–	–	8.60 ± 0.20	10.60 ± 0.20
Yield (%)	–	–	44.99 ± 5.10	55 ± 5.42

Average values (*n* = 3) ± standard deviation

EC electrical conductivity; – no value

Results

Effects on soil physicochemical properties by amending with modified and non-modified biochar

Soil AP and AK levels were significantly influenced by soil type, WBC and Mg-WBC addition, and their interaction (soil × treatment). In QY soil, the amendments with each of the 1% WBC and Mg-WBC did not show significant differences for soil AP as regards to these treatments, while the differences were significant when compared with CK (Fig. 2a). Contrastingly, the amendments with 2.5% WBC and Mg-WBC showed significant differences. In the case of soil HAR, significant differences were found for all biochar rates. The AP concentration in QY increased by 63%,154.5%, 71%, and 229% for WBC_1, $WBC_{2.5}$, Mg-WBC_1 and Mg-$WBC_{2.5}$, respectively, while for HAR the increases were 56%, 139%, 64% and 147%, respectively, as compared to CK. The AK concentration in QY was significantly enhanced by either rate of WBC or Mg-WBC amendments, as compared to CK (Fig. 2b). The highest AK concentration (252 mg kg^{-1}) was associated with the Mg-$WBC_{2.5}$ treatment, while the lowest (90 mg kg^{-1}) corresponded to CK in QY. Compared with CK, the AK concentration in HAR was significantly increased

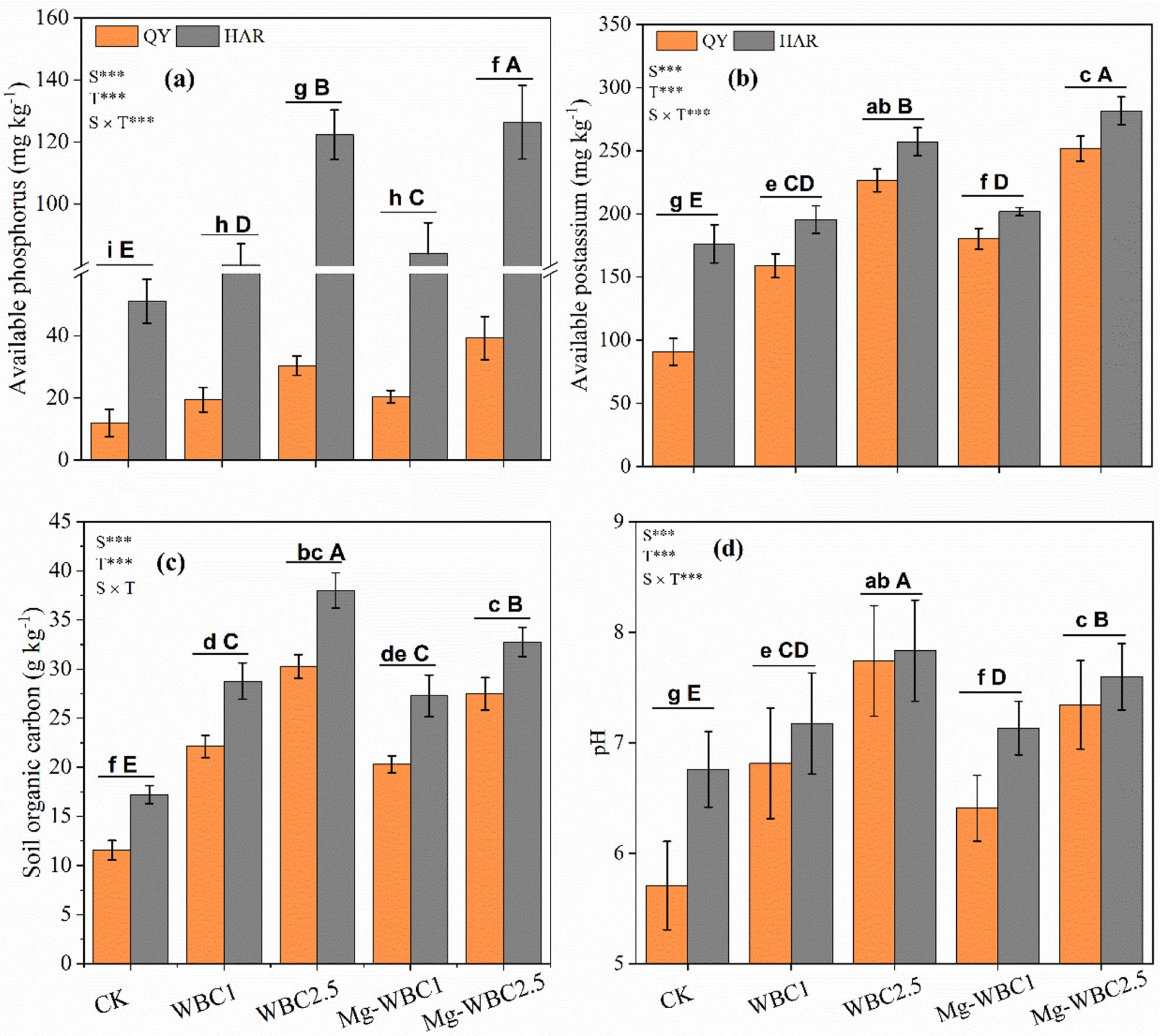

Fig. 2 Effect of WBC and Mg-WBC on available phosphorus (**a**), available potassium (**b**), soil organic carbon (**c**), and pH (**d**). QY and HAR represent: Qiyang red soil and Harbin black soil, respectively. Different small (represent QY) and capital (represent HAR) letters show statistically significant differences between the treatments of both soils (soil x treatment) ($p < 0.05$), and error bars are the standard deviation of the mean ($n = 3$)

after the addition of both biochar materials. AK concentration in HAR increased by 10%, 45.8%, 14.4% and 60%, for WBC_1, $WBC_{2.5}$, $Mg\text{-}WBC_1$ and $Mg\text{-}WBC_{2.5}$, respectively, when as compared to CK.

Similarly in case of SOC and soil pH, both were significantly enhanced by biochar addition, soil type, but the interaction (soil × treatment) was only significant for soil pH. The $WBC_{2.5}$ amendment significantly increased SOC concentration under both soils, when compared with CK and the lower rate (1%) of both WBC and Mg-WBC (Fig. 2c). Under $WBC_{2.5}$ treatment, the maximum SOC value was 161% in QY, whereas it reached 120% in HAR, when compared with their respective control. The pH values showed an increase for both QY and HAR soils after the WBC and Mg-WBC amendments at either rate of biochar, as compared to CK (Fig. 2d). Moreover, 1% dose of both biochar materials did not cause significant differences within the treatments in HAR soil and showed significant variation in QY soil, while the 2.5% rate was associated with significant differences among biochar treatments and CK.

Effects of modified and nonmodified biochar on soil microbial biomasses

The MBC and MBP were significantly increased in QY and HAR soils after the addition of WBC and Mg-WBC at both rates (1% and 2.5%) as compared to CK, while the interaction (soil x treatment) was only significant for MBP. The highest (262 mg kg^{-1}) MBC level in QY was recorded for the $WBC_{2.5}$ amendment, while the lowest (236 mg kg^{-1}) corresponded to CK. In the HAR soil, MBC increased after biochar addition when compared with the control, with an increasing trend like that of QY following the order: $Mg\text{-}WBC_{2.5} = WBC_{2.5} > Mg\text{-}WBC_1 = WBC_1 > CK$ (Fig. 3a). In QY, the 2.5% dose of both biochar materials was associated with a significantly higher concentration of MBP when compared with the 1% dose (Fig. 3 b). MBP increased by 23%, 52%, 29%, and 62% in WBC_1, $WBC_{2.5}$, $Mg\text{-}WBC_1$ and $Mg\text{-}WBC_{2.5}$, respectively, when compared to CK. In HAR, MBP concentration significantly increased due to all biochar treatments. In addition, both the 1 and 2.5% doses were associated with significant changes when compared with CK. The 1% and 2.5% rate caused an increase (34–44% and 63–75%, respectively) in MBP concentration when compared with CK. In HAR soil, the highest MBP concentration (48 mg kg^{-1}) was observed for the $Mg\text{-}WBC_{2.5}$ treatment, while the lowest (27 mg kg^{-1}) was that of CK.

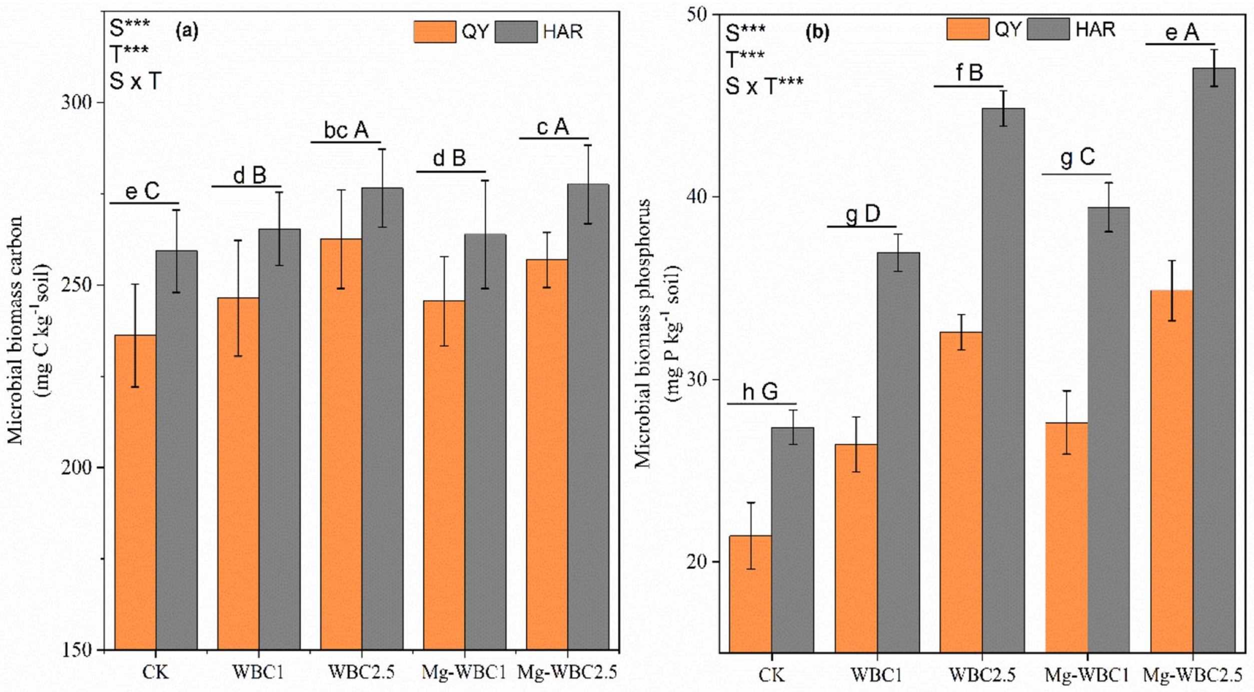

Fig. 3 Effect of WBC and Mg-WBC on MBC (**a**) and MBP (**b**). QY and HAR represent Qiyang red soil and Harbin black soil, respectively. Different small (represent QY) and capital (represent HAR) letters show statistically significant differences between the treatments of both soil (soil x treatment) ($p <$ 0.05), and error bars are the standard deviation of the mean ($n = 3$)

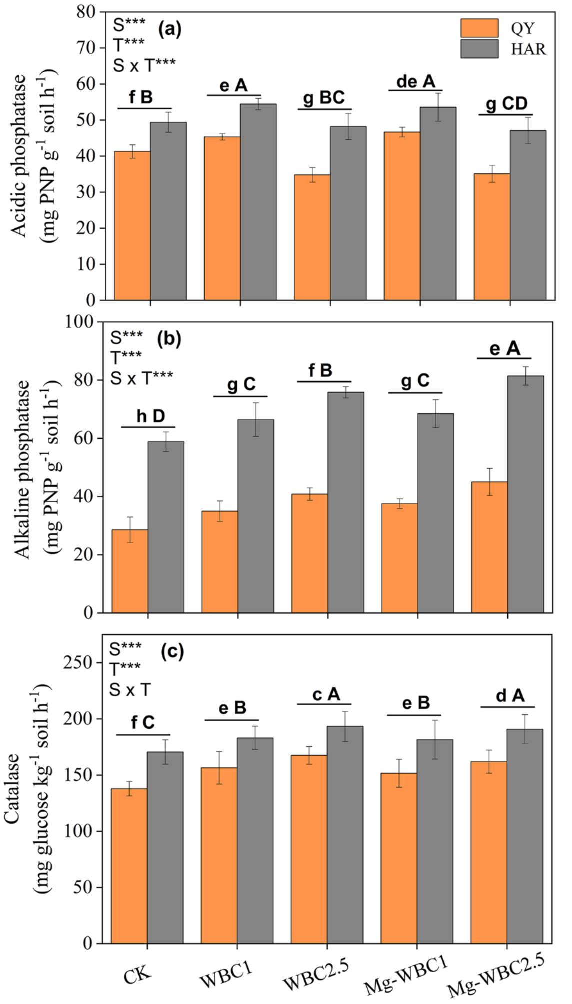

Fig. 4 Effect of WBC and Mg-WBC on acidic phosphatase (**a**), alkaline phosphatase (**b**), and Catalase (**c**). QY and HAR represent Qiyang red soil and Harbin black soil, respectively. Different small (represent QY) and capital (represent HAR) letters show statistically significant differences between the treatments of both soil (soil x treatment) ($p <$ 0.05), and error bars are the standard deviation of the mean ($n = 3$)

Impact of modified and nonmodified biochar on soil enzymatic activities

Enzymatic activities of acidic phosphatase (AcP), alkaline phosphatase (AlP) and catalase (Cat) were significantly enhanced by soil type, biochar addition and their interaction, except Cat which revealed nonsignificant (soil ×treatment) interaction. The AcP activities in QY and HAR soils were significantly influenced by the biochar rates (Fig. 4a). Compared with CK, 1% of WBC and Mg-WBC dose increased AcP activity, while it decreased at 2.5% dose in both soils. For soil QY, the AcP activity increased by 10% and 13% in WBC_1 and Mg-WBC_1, while it decreased by 15% and 14% in $WBC_{2.5}$ and Mg-$WBC_{2.5}$, respectively. Similarly, in HAR the 2.5% rate of both biochar materials triggered a decrease in the AcP activity, compared to the 1% dose and to CK. In HAR, the highest AcP activity (54 mg PNP g^{-1} soil h^{-1}) was observed for the WBC_1 treatment and the lowest (47 mg PNP g^{-1} soil h^{-1}) for Mg-$WBC_{2.5}$.

Compared with CK, the biochar treatments significantly increased the AlP activity (Fig. 4b) in QY and HAR. In QY, the AlP increased by 18%, 29%, 23%, and 36% in WBC_1, $WBC_{2.5}$, Mg-WBC_1 and Mg-$WBC_{2.5}$, respectively, as compared to CK. In HAR, the AlP revealed no significant difference at 1% dose of both biochar materials within the treatment, while differences were significant within the treatment for the 2.5% rate. The AlP increased by 39% for Mg-$WBC_{2.5}$, followed by 35% for $WBC_{2.5}$, 28% for Mg-WBC_1, and 26% for WBC_1, when compared to CK.

The catalase activity in QY soil did not show any significant differences at 1% rate of WBC and Mg-WBC within the treatment, whereas the 2.5% revealed higher and significantly different activity compared with CK (Fig. 4c). In QY, the highest catalase activity (167 mg glucose kg^{-1} soil h^{-1}) corresponded to $WBC_{2.5}$, while the lowest (137 mg glucose kg^{-1} soil h^{-1}) was that of CK. Similarly, in HAR soil, catalase activity was also improved by the WBC and Mg-WBC amendments at either level, as compared to CK, although the individual 1% and 2% doses of WBC and Mg-WBC revealed nonsignificant differences. The catalase activity was increased by 7–11% in WBC and by 6–10% in Mg-WBC in HAR.

Effects of modified and nonmodified biochar on soil phosphorus fractionation

All the P fractions were significantly influenced by both biochar materials and soil type (Table 2). The resin-P concentrations were higher in Mg-$WBC_{2.5}$ treatment in both soils. $NaHCO_3$-Pi showed significant differences, while $NaHCO_3$-Po exhibited low differences, although with higher concentration for $WBC_{2.5}$ in QY, while in HAR soil, Mg-$WBC_{2.5}$ showed higher levels when compared to the control. Owing to the Mg-$WBC_{2.5}$ amendment, resin-P increased by 114% and 28% in QY and HAR soil, respectively, while $NaHCO_3$-Pi and Po increased by 81% and 11% for $WBC_{2.5}$ in QY soil and by 77% and 37% in HAR soil for Mg-$WBC_{2.5}$, as compared to CK. The WBC_1 treatment decreased NaOH-Pi and Po under both soils, specifically by 26% and 43% in QY, and by 31% and 39% in HAR, while it increased Dil-HCl-P in $WBC_{2.5}$ by 51% and 50% in both QY and HAR soils when compared with CK. The concentration of Con-HCl-Pi and residual-P significantly decreased with the highest application rate of both biochar while Con-HCl-Po showed no effect. For Mg-$WBC_{2.5}$, Con-HCl-Pi and residual-P decreased by

Table 2 Values showing the effect of WBC and Mg-WBC on phosphorus (P) fractionation (results expressed in mg kg^{-1}) in QY and HAR soil, under different biochar treatments. Average values (n = 3) ± standard deviation

Sites	Treatments	Labile P mg kg^{-1}			Moderately labile P mg kg^{-1}			Nonlabile P mg kg^{-1}		
		Resin-P	**NaHCO$_3$ -Pi**	**NaHCO$_3$ -Po**	**NaOH-Pi**	**NaOH-Po**	**Dil-HCl-P**	**Con-HCl-Pi**	**Con-HCl-Po**	**Residual-p**
QY	CK	50.9 ± 5.9 f	62.3 ± 8.3 e	11.5 ± 0.9 c	67.2 ± 3.1 ef	26.1 ± 2.6 a	85.1 ± 3.8 f	104.6 ± 6.6 d	14.7 ± 1.5 bc	89.4 ± 6.5 b
	WBC$_1$	82.3 ± 6.1 e	95.3 ± 12.8 d	10.5 ± 1.3 c	58.1 ± 2.5 g	14.8 ± 0.5 bcd	99.4 ± 6.4 e	87.8 ± 5.1 e	12.6 ± 2.7 c	70.5 ± 4.9 c
	WBC$_{2.5}$	101.7 ± 8.3 d	113.3 ± 7.5 b	12.9 ± 1.6 c	49.3 ± 5.1 h	21.8 ± 1.5 ab	128.1 ± 5.3 c	78.1 ± 6.1 f	10.5 ± 2.9 c	58.2 ± 5.2 de
	Mg-WBC$_1$	87.1 ± 7.8 e	101.7 ± 6.2 cd	9.6 ± 0.7 c	59.7 ± 3.9 fg	18.1 ± 4.4 abc	95.6 ± 2.6 e	88.5 ± 5.9 e	11.8 ± 4.1 c	73.1 ± 6.8 c
	Mg-WBC$_{2.5}$	108.7 ± 8.5 cd	110.9 ± 7.6 bc	12.1 ± 1.2 c	54.4 ± 4.6 gh	24.4 ± 3.5 a	115.9 ± 5.4 d	71.4 ± 2.9 f	9.9 ± 1.2 c	55.4 ± 5.1 e
HAR	CK	108.6 ± 8.3 cd	72.5 ± 4.6 e	17.1 ± 4.7 b	107.1 ± 8.2 a	12.3 ± 3.1 de	104.2 ± 4.7 e	139.1 ± 5.3 a	25.6 ± 4.1 a	126.9 ± 4.2 a
	WBC$_1$	118.6 ± 7.1 bc	90.9 ± 4.7 d	18.6 ± 1.2 b	89.4 ± 5.2 b	10.3 ± 1.7 def	116.6 ± 2.8 d	127.7 ± 4.1 b	21.1 ± 1.8 ab	98.1 ± 5.7 b
	WBC$_{2.5}$	131.9 ± 6.9 ab	112.3 ± 4.5 bc	23.1 ± 3.3 a	73.1 ± 3.9 de	7.4 ± 0.8 f	156.8 ± 3.1 a	113.5 ± 6.5 c	22.4 ± 2.2 a	67.2 ± 2.2 cd
	Mg-WBC$_1$	124.7 ± 3.9 b	97.3 ± 2.7 d	18.4 ± 1.2 b	84.2 ± 2.4 bc	11.8 ± 0.9 def	114.6 ± 4.1 d	120.2 ± 5.8 bc	20.7 ± 1.6 ab	92.9 ± 2.6 b
	Mg-WBC$_{2.5}$	139.5 ± 3.9 a	129.1 ± 3.5 a	23.4 ± 3.5 a	76.3 ± 5.8 cd	8.1 ± 1.6 ef	145.5 ± 8.3 b	102.3 ± 6.6 d	22.1 ± 1.1 a	63.3 ± 4.3 cde
ANOVA										
S		***	ns	***	***	***	***	***	***	***
T		***	***	**	***	**	***	***	ns	***
S*T		*	*	ns	*	**	ns	ns	ns	**

QY Qiyang soil, *HAR* Harbin soil, *Pi* Inorganic P fraction, *Po* Organic P fraction, *Dil-HCl-P* Dilute HCl P fraction, *Con-HCl-P* Concentrated HCl P fraction, *CK* control, *WBC$_1$* Raw biochar added into soil at 10 g kg^{-1}; WBC$_{2.5}$: Raw biochar added into soil at 25 g kg^{-1}; Mg-WBC$_1$: Modified biochar added into soil at 10 g kg^{-1}; Mg-WBC$_{2.5}$: Modified biochar added into soil at 25 g kg. *** ($P \leq 0.0001$), ** ($P \leq 0.001$), * ($P \leq 0.05$), ns (no significant differences)

31–38% in QY, and by 26%,—50% in HAR, respectively, when compared with control.

WBC and Mg-WBC significantly affected the soil P pools in QY and HAR, while the interaction (soil × treatment) was only significant for LP (Fig. 5). Compared with CK, the biochar application to these soils (QY and HAR) strongly increased the labile P (LP) and slightly increased the moderately labile P (MP), while it decreased the nonlabile P (NP). The increases for LP were 50%, 82%, 59%, and 85% in QY soil and 15%, 34%, 21%, and 47% in HAR soil, for WBC_1, $WBC_{2.5}$, Mg-WBC_1, and Mg-$WBC_{2.5}$, respectively. The 1% dose of both biochar materials decreased MP by 2–3% in QY soil, and by 3–6% in HAR soil, while at the dose of 2.5% it slightly increased MP, specifically by 9–11% in QY soil, and by 3–6% in HAR soil. The NP concentrations significantly decreased under both soils at either level of WBC or Mg-WBC. The percent decreases were 18%, 29%, 17%, and 34% in QY soil, while it was 15%, 30%, 19%, and 35% in HAR soil, this taking place for WBC_1, $WBC_{2.5}$, Mg-WBC_1, and Mg-$WBC_{2.5}$, respectively.

Relationships between biochemical properties and P fractions

The Mantel analysis (Fig. 6) showed that soil properties (AP, AK, SOC, pH), soil enzymes (AcP, AlP, Cat), and

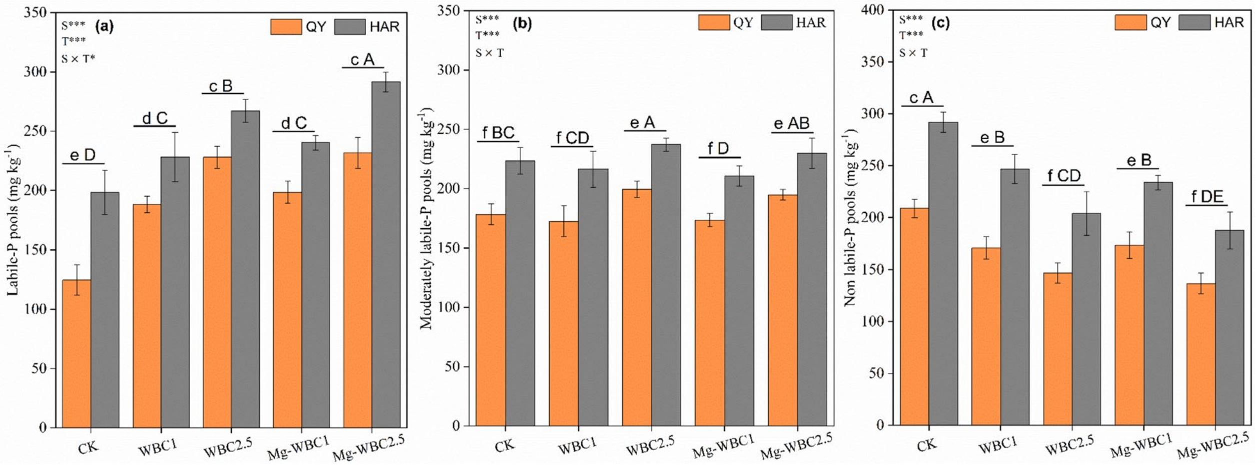

Fig. 5 Impact of WBC and Mg-WBC on **a** labile, **b** moderately labile and **c** nonlabile phosphorus pools

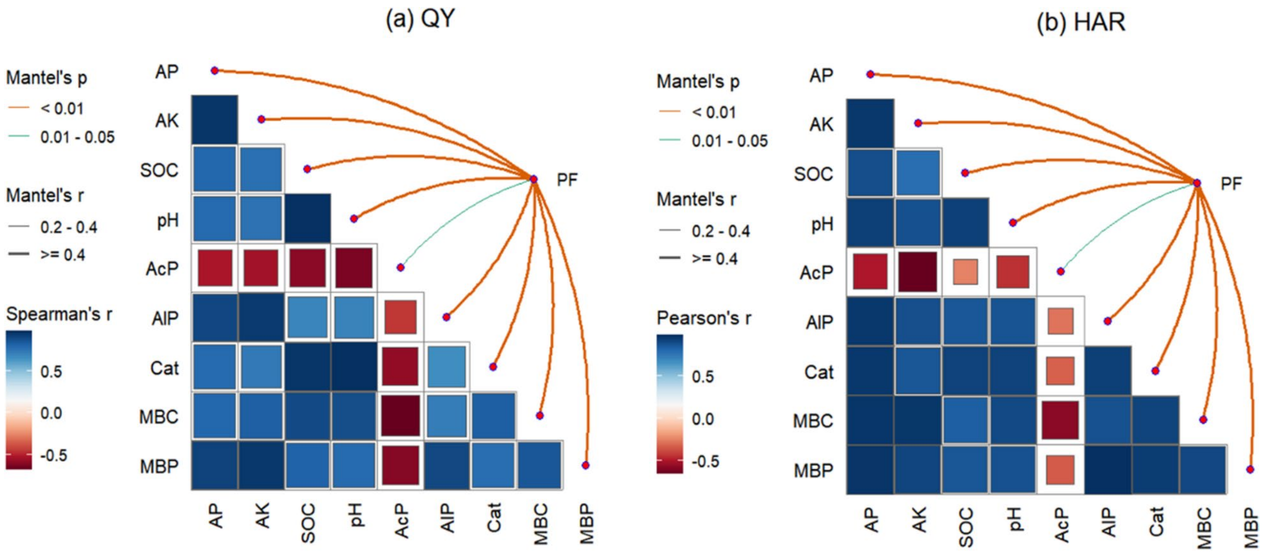

Fig. 6 Relationships among soil properties, microbial biomass, enzymatic activities and soil P fractions in QY and HAR soils according to the Mantel's test

microbial biomass (MBC and MBP) significantly correlated with P fractions (PF). Through the Mantel correlation coefficients, it was shown that SOC has a stronger effect on QY soil, while AP and MBC have a stronger effect on HAR soil.

The PLS-PM was used to further investigate the direct and indirect effect of soil properties (SP: AP, AK, SOC, and pH), soil enzymes (SA: AcP, AlP, and Cat) and microbial biomass (MBC, and MBP) on P fractions (PF: LP, MP, and NP). When both biochar materials (WBC and Mg-WBC) were compared for QY and HAR soils, it was shown that SP significantly and directly affected PF in both QY and HAR soils, with path coefficient (β) values of 0.102 and 0.414, respectively. Similarly, under the WBC amendment, SP indirectly affected PF, with a path coefficient of $\beta = 0.483$, which was mediated by MB, and soil enzymes presented nonsignificant differences. In the case of Mg-WBC, MB exhibited a significantly positive direct effect on PF ($\beta = 1.379$), while soil enzymes showed a significantly negative direct effect on PF ($\beta = -0.117$). Moreover, SP showed a significantly indirect effect on PF concentrations, which was mediated by MB and soil enzymes, with path coefficients $\beta = 0.981$ and 0.314, respectively, while the GOF values of both biochar materials were 0.569 for WBC, and 0.885 for Mg-WBC, confirming that Mg-WBC improved P availability under acidic to neutral soils, this was achieved by converting organic P into inorganic P (plant available P).

Different small (represent QY) and capital (represent HAR) letters show statistically significant differences between the treatments of both soil (soil x treatment) ($p < 0.05$), and error bars are the standard deviation of the mean ($n = 3$).

Discussion

Response of soil available P and K to modified and nonmodified biochar amendments

The addition of WBC and Mg-WBC significantly increased AP and AK concentrations in both soils. Furthermore, AP and AK levels were higher in HAR compared to QY soil, which is due to the initial higher values in HAR. The AP increase after the WBC and Mg-WBC amendments (especially at the 2.5% rate) would be due to the presence of unstable organic compounds in the amendments, which would rise AP availability [32]. The increase in soil pH with biochar addition is also one of the main reasons for P availability. In acidic soil, biochar (rising pH) shift the formation of insoluble Fe–P and Al-P into plant available forms [4]. Furthermore, it increased indirectly the P availability, decreasing the positive charge on Fe and Al oxides, having lower affinity to adsorb negatively charged PO_4^{3-} [7]. The increase in soil AK could be related to the ash content in the biochar amendments. Table 1 showed that modified biochar with a higher total K content caused a significant rise in AK content for Mg-WBC$_{2.5}$, particularly in QY soil which was initially poorer in exchangeable K than HAR soil. It was reported that biochar may increase AK concentrations due to easily release of this nutrient into the soil solution [33, 34]. Moreover, AP and AK concentrations in the current study increased as biochar rate increases, which is in accordance with the previous studies [23, 35]. Another possible reason for the increase in nutrient concentration after biochar amendment (specially for AP and AK) was due to reduced nutrient leaching [32], as biochar has a high surface area and negative charge, which increase soil cation exchange capacity and subsequently the availability of soil nutrients [36, 37].

Response of soil organic C pH to modified and nonmodified biochar

SOC increased in both soils (QY and HAR) after the addition of WBC. Specifically, SOC concentrations showed an overall rise as a function of biochar rate, probably due to the stability and recalcitrant nature of the material, although the increased SOC would not be available for soil microbes [38]. However, a small decrease in SOC concentration was found after amending with Mg-WBC, as the incorporation of Mg into carbon pore spaces makes this modified material more stable when compared with nonmodified biochar [39]. The mineral–organic interaction on biochar surface helps in the formation and stabilization of soil aggregates which further protect the SOC from microbial degradation [40]. The biochar particle size also influences SOC concentration. Biochar with smaller particle sizes (< 2 mm) has higher surface area (< 1 mm used in the current study), which increases the adsorption of organic C on its surface and thus, SOC retention capacity increased [40, 41]. Similarly, Bashir et al. [41] also reported an increase in SOC concentration after biochar amendment and correlated their results with the higher surface area and porous structure of the biochar used.

The pH values were significantly increased in both soils as a function of WBC dose, mainly due to the alkaline nature of biochar [42] and the presence of basic cations in this material, as shown by the EDS analyses (Fig. S1). These results are in line with those from Sial et al. [43] and Sui et al. [1]. However, a slight decrease in pH was observed in Mg-WBC treatments, due to the initial lower pH of the modified material (Table 1). This was related to the $MgCl_2$ modification. After the pyrolysis, HCl was produced, then decreasing the pH of the resulting biochar [39]. The effect could be different using other biochar modifiers, as noted by Wang et al. [44], who reported an increase in pH values after the application of Fe–Mn-modified biochar to a slightly alkaline soil.

Response of soil microbial biomass to modified and nonmodified biochar

Soil microbial biomass significantly increased under biochar treatments at either rate, when compared to CK. The increase in MBC and MBP after biochar amendments would be due to: (a) biochar addition provides more favorable conditions for soil microbes by increasing pH (which reduces Al toxicity on microbes and ultimately causes MBC and MBP to increase [45]); (b) the biochar content in labile organic C is used by microbes as source of energy, rising soil microbial biomass [46, 47]; and (c) the high surface area and porous structure of biochar, which provide shelter for microbes, as well as promote water and nutrient adsorption on its surface, finally increasing microbial growth and biomass [48, 49]. These results are supported by Mantel's correlations and PLS-PM analyses (Figs. 6 and 7), specifically showing significant positive relationship for soil pH and SOC with MBC and MBP. Also relevant, the MBC concentration in QY soil (which has pH 5.6) was significantly increased as compared to HAR soil (with pH 6.7). These results are in line with the meta-analysis by Pokharel et al. [50]. Furthermore, in the current study, the amendment of Mg-WBC promoted higher MBP rise than WBC, as Mg-WBC increases P availability, which was previously reported by Li et al. [15].

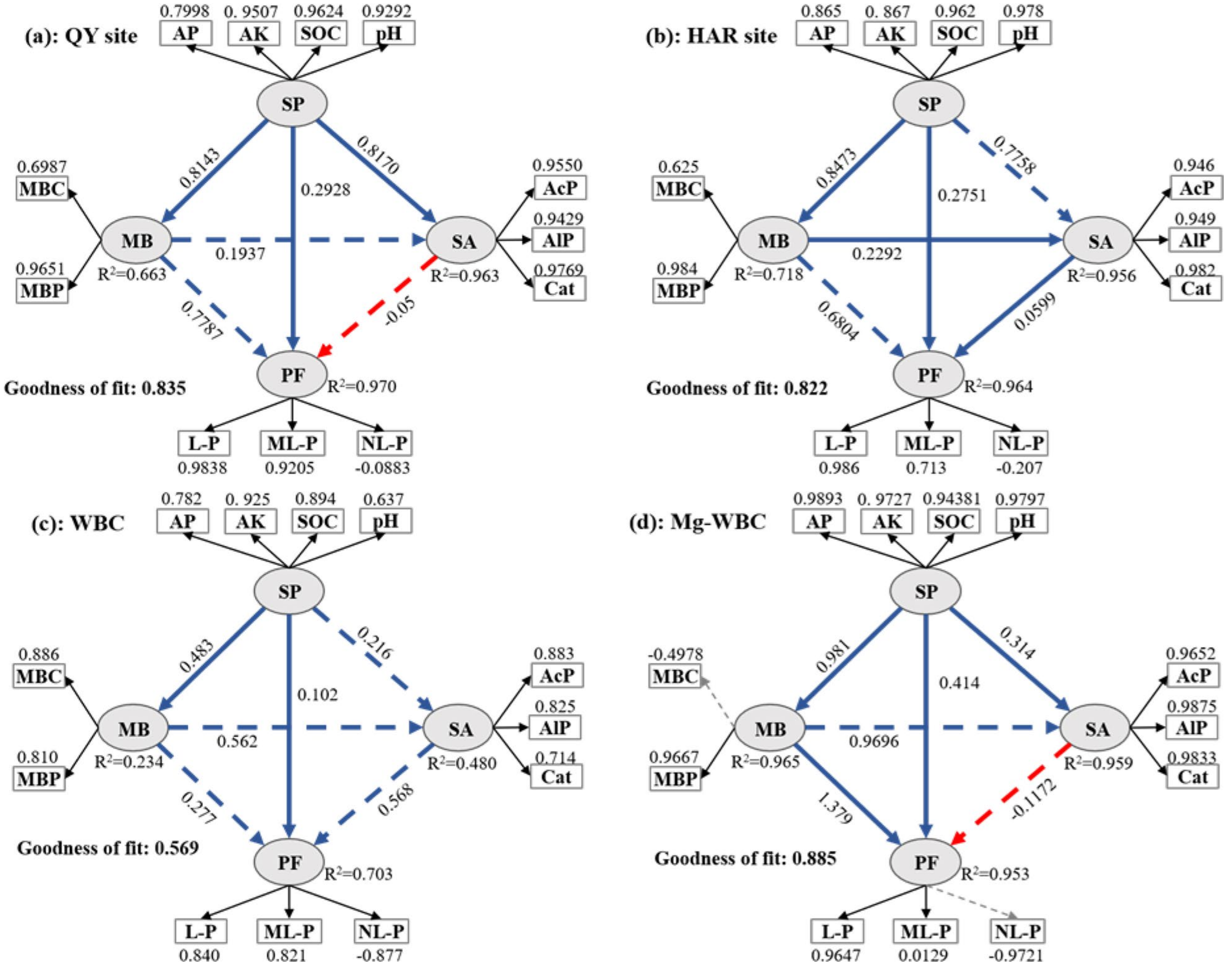

Fig. 7 Partial least-square path model (PLS-PM) representing the direct and indirect effect of soil properties (AP, AK, SOC and pH), soil microbial biomass (MBC and MBP), and enzymatic activities (AcP, AlP, and Cat) on phosphorus fractions (LP, ML-P, and NL-P); **a** and **b** display the PLS-PM analysis based on the site (QY and HAR). **c** and **d** show the PLS-PM analysis based on the biochar types (WBC and Mg-WBC). *AP* Available phosphorus, *AK* available potassium, *SOC* soil organic carbon, *MBC* and *MBP* microbial biomass carbon and phosphorus, *AcP* and *AlP* acidic and alkaline phosphatase, *Cat* catalase, *LP* labile P fraction, *ML-P* moderately labile P fraction, *NL-P* nonlabile-P

Response of soil enzymatic activities to modified and nonmodified biochar

Phosphatase activities were significantly increased depending on the rate of biochar amendment. The increasing trend showed by AlP (especially at the 2.5% rate) could be mainly attributed to the rise in pH, with similar results being reported by Jain et al. [51]. The other possible reason for the rise in AlP activity was the increase in Mg ions (Mg-modified biochar used in this study) and other coenzyme factors of AlP [52]. Furthermore, the AlP showed positive correlation with MBC and MBP (Fig. 6), as previous found by Peng et al. and Palansooriya et al. [52, 53]. However, at the opposite, the acidic phosphatase (AcP) activity was decreased after the addition of WBC and Mg-WBC at a higher rate. This decrease was more pronounced at the highest rate of both WBC and Mg-WBC (2.5%). Likely due to the alkaline nature of the biochar [50], H^+ (proton) ion concentrations lower, thereby resulting in a decrease of AcP activity [52]. The other reason was the increase in P availability after biochar addition, as the higher P availability may lead to the suppression of Fe ion, catalyzing phosphatase reaction, and resulting in a decrease of AcP activity [54].

In the present research, catalase activity significantly increased with the addition of WBC and Mg-WBC. It should be noted that the catalase activity is mostly studied in relation to that of microbes and its population in soils [55], supported by the positive correlation of soil properties (as well as microbial biomass, MBC and MBP) with catalase activity (Fig. 6). Wojewódzki et al. [56] as well as Nie et al. [57] also observed a significant increase in enzymatic activity after a biochar amendment. Furthermore, previous research [58, 59] has shown that the activity of soil enzymes would be affected by the surface area of biochar, mainly due to its retention potential and subsequent capacity for changing or rotating the active site of enzymes.

Effect of modified and nonmodified biochar on soil phosphorus fractions

In QY and HAR soils, the labile P (resin-P and $NaHCO_3$-P) fractions were significantly improved after WBC and Mg-WBC addition. When compared with the biochar type, the Mg-WBC showed significant enhancements in labile P (primarily for HAR with only minor changes for QY), due to the presence of Mg ions that bind with P to form Mg-P (with weak chemical bonds), which is more soluble than Fe–P and Al-P (P makes insoluble complexes with Fe and Al in acidic soil) [60]. The other possible reason for the increase in labile P was biochar high surface area and alkaline nature, increasing the pH of acidic soils and making the P available that linked to soil colloids or Al and Fe in acidic edaphic environment [1, 47]. The PLS-PM analysis also showed a good model fit for QY (0.83) when compared to HAR (0.822), indicating that biochar presented better results for more acidic soils (Fig. 7 a). The low organic-P concentrations found in the current study indicated that the P is transformed into plant available P forms (Pi), increasing labile P fractions. In this regard, Glaser and Lehr [61] reported that biochar application increased labile P in acidic (pH <6.5) and neutral soils (pH 6.5–7.5), while nonsignificant effect was found in alkaline soils. In the current investigation, Dil-HCl-P increased depending on the biochar rate. To note that Dil-HCl-P mainly determined P fixation with Ca, and both QY and HAR soils increased their pH especially due to the concentration of Ca applied with the biochar amendments, then promoting the subsequent binding to P [2, 62].

The moderately-labile (NaOH-Pi), nonlabile (Con-HCl-Pi) and residual-P significantly decreased with the addition of WBC and Mg-WBC, whereas little changes were observed for NaOH-Po and Con-HCl-Po, suggesting that biochar stimulated the decomposition of organic matter and favored the dissolution of nonlabile-P (Ca-bound-P) and residual-P into plant available P [63]. Moreover, the Mg ions in the Mg-WBC interact with Fe and Al in the moderately and nonlabile P (Fe–P and Al-P) converting those stable P complexes into plant available form (Mg-P). Secondly, the modified biochar reversibly adsorb phosphate ions and add to labile P [64]. In a previous study, [1] showed that the addition of biochar to a black soil significantly increased the concentration of labile-P and Dil-HCl-P fractions, while decreased the nonlabile and residual P. The decrease in nonlabile and residual P was mainly due to the fact that biochar caused a rise in the labile P fractions [2] and biochar application into the soil mainly acted on the inorganic P fractions, which ultimately increased P availability [1]. From the above discussion, we conclude that Mg-WBC performs well for increasing P availability and soil health in both QY and HAR soils, when compared with WBC. In addition, the WBC improves soil structure and water retention, but due to negative charge, it has little affinity to attract PO_4^{3-}. Therefore, modification of biochar is a good strategy to increase the positively charged sites on biochar surface to adsorb PO_4^{3-} and make it available for plant uptake.

Conclusions

Overall, the Mg-WBC biochar studied here can be considered as an alternative to improve the physicochemical and biological properties of soils, by increasing the concentration and availability of soil nutrients and labile C and assisting the remediation of acidic soil, which would be the key for achieving a subsequent and sustainable increase in productivity for farmers using these resources. Furthermore, both WBC and Mg-WBC significantly increased P availability and could reduce the loss of cations and anions in/from acidic to neutral soils. The application of Mg-WBC significantly increased the concentration of labile P and Dil-HCl-P, while decreased the moderately- and nonlabile P concentrations, revealed by PLS-PM analysis. The application of Mg-WBC to acidic soils increased soil pH, enhanced the formation of Fe and Al complexes with P and increased the dissolution of nonlabile and residual P to improve plant available P concentrations. As specifically highlighted above regarding productivity and sustainability, these results can be seen as relevant from both the agronomic and environmental perspectives. In addition, future research could be focused on an in-depth assessment of molecular basis related to the effect of modified biochar on P availability.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-025-01128-3.

Supplementary Material 1.

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Author contributions
Muhammad Numan Khan: Conceptualization, Data curation, Formal analysis, methodology, writing original draft, Writing—review & editing. Huimin Zhang and Liu Wenjie: Conceptualisation, Resources, Writing—Review & editing. Jing Huang, Asad Shah, and Imtiaz: Data curation, methodology. Tainfu Han and Avelino Núñez-Delgado: Data curation, investigation, methodology. Hao Xiaoyu: Formal analysis, Resources. Nafiu Garba Hayatu: Data curation, Resources. Ashutosh Kumar Singh: Investigation, Resources. Xiai Zhu: Formal analysis, Methodology.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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