



# Biochar Alters Inorganic Phosphorus Fractions in Tobacco-growing Soil

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Received: 23 November 2020 / Accepted: 31 March 2021 / Published online: 27 April 2021  
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## Abstract

It is important to consider tobacco stalks as sources of biochar and improve the soil quality of tobacco-producing areas in Southwest China. Therefore, in this study, we investigated the effects of biochar produced from tobacco stalks on soil inorganic phosphorus (P) fractions in bulk and rhizosphere soils. We also analyzed the factors influencing these effects in tobacco-planting fields. Biochar was applied to two experimental fields in Heishi and Qianxi in Bijie City, Guizhou Province in China, at application rates of 0, 5, 20, and 40 t ha<sup>-1</sup>. Subsequently, the soil inorganic P (P<sub>i</sub>) fractions, pH, and exchangeable and hydrolytic acidities were analyzed, while the factors influencing the soil P<sub>i</sub> fractions were determined by redundancy analysis (RDA). The results indicated that the biochar application rate, experimental site, and tobacco roots all affected the soil P<sub>i</sub> fractions. Aluminum-phosphate (Al-P), iron-phosphate (Fe-P), and occluded-phosphate (O-P) levels in the bulk soil had significantly increased with biochar application in the Qianxi site. Meanwhile, there was a reduction in Al-P, Fe-P, and O-P levels in the rhizosphere soil in the Heishi site but not in the Qianxi site. The calcium-phosphate (Ca-P) levels in the soil were also greatly reduced in the Heishi site, while in the Qianxi site, the Ca-P levels were relatively constant in the bulk soil and increased in the rhizosphere soil. Furthermore, the exchangeable and hydrolytic acidities decreased with increasing biochar application. Soil P<sub>i</sub> fractions varied according to biochar application rate and experimental site. We found that 20 t ha<sup>-1</sup> was an appropriate biochar application rate for enhancing soil P<sub>i</sub> fractions. In addition, the soil P<sub>i</sub> fractions were negatively correlated with the dissolved organic carbon (DOC) content but were positively correlated with the hydrolytic acidity.

**Keywords** Tobacco stalk biochar · Inorganic phosphorus fractions · Soil acidity · Tobacco-growing field

## 1 Introduction

Phosphorus (P), a nonrenewable resource, is necessary for crop growth and development. However, due to strong fixation in the soil, the availability of soil P is very low (Zheng

**Highlights** • Soil inorganic phosphorus (P) fractions depend on biochar application rate and experimental site.

- Soil inorganic P (P<sub>i</sub>) fractions significantly correlated with dissolved organic carbon content and hydrolytic acidity.
- Tobacco stalk biochar application at 20 t ha<sup>-1</sup> was appropriate for enhancing soil P<sub>i</sub> fractions.

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et al. 2019). In acidic soils, P is usually fixed by iron (Fe), aluminum (Al), and their oxides to form insoluble P in forms such as iron-phosphate (Fe-P), aluminum-phosphate (Al-P), and occluded-phosphate (O-P). Meanwhile, in alkaline soils, it is adsorbed or precipitated by calcium (Ca) to form calcium-phosphate (Ca-P) (Baumann et al. 2017; Cao et al. 2020a). Consequently, the availability of P is relatively high only when the soil pH is approximately 6–7. The P<sub>i</sub> fractions combined with Fe, Al, and Ca are not easily used by plants; however, soil Al-P, Fe-P, and some of the Ca-P can be converted into free phosphate, which provide an important P pool for plant growth and development (Liu et al. 2019). In acidic soils, Al-P and Fe-P are labile P<sub>i</sub> fractions for crops (Ch'ng et al. 2014a, b). The precipitation and dissolution of P are reversible; therefore, when the hydroxide ion (OH<sup>-</sup>) concentration of these fixed P sources increases in the soil, strengite and phosphorite can dissolve and release P. Moreover, increased OH<sup>-</sup> concentrations in the soil can reverse the exchange of dihydrogen phosphate (H<sub>2</sub>PO<sub>4</sub><sup>-</sup>) and OH<sup>-</sup> on the

surface of the soil minerals, re-releasing  $\text{H}_2\text{PO}_4^-$ . The solubility of the gel film on the surface of O-P molecules is extremely low, resulting in insufficient availability of O-P for plants.

Various measures, such as phosphate-dissolving microbes (Bargaz et al. 2018), breeding (van de Wiel et al. 2016) and others, have been used for enhancing soil P availability and improving plant P levels. Among these measures, the use of biochar has been reported to increase soil nutrient availability (Scott et al. 2014; Xu et al. 2013) and improve soil P nutrition (Jin et al. 2016; Parvage et al. 2013; Zhai et al. 2015). Biochar is produced via the pyrolysis of biomass residues in an oxygen-deficient and high-temperature environment, and is widely used as a soil amendment and important bio-resource (Kwon et al. 2020). It has been reported that rice-residue biochar can increase both P availability and P use efficiency of the applied or reserve P to fulfill the present P demand (Mukherjee et al. 2020). Biochar increases the available  $\text{P}_i$  fractions of the soil and can maintain high soil P content for at least 2 years after its application (de Figueiredo et al. 2020). Furthermore, biochar has high P concentration in its ash, and can directly release soluble P while improving P availability to plants (Wang et al. 2012; Zhai et al. 2015; Borges et al. 2020). Moreover, when added to the soil, biochar can also improve its physical, chemical, and biological properties, thereby indirectly affecting P availability (Gao and DeLuca 2020; Zhou et al. 2020). Biochar is generally alkaline, and when applied to acidic soil it can reduce soil acidity and the fixation of P by Fe and Al cations ( $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$ ) (Wang et al. 2012). Cui et al. (2011) demonstrated that in the presence of biochar, the surface P adsorption and the adsorption rate of ferrihydrite were reduced; however, when ferrihydrite was combined with biochar, the desorption performance improved. Therefore, biochar can reduce the adsorption of P by Fe oxides and enhance the P availability in acidic soils. In addition, the application of biochar to acidic soil (Ultisols) may increase the content of Al-P and Ca-P, and reduce the content of Fe-P (Hong and Lu 2018). Other studies have also found that biochar application to acidic soils can increase the content of Ca-P and P extracted by potassium chloride (KCl-P) and the availability of P and reduce the content of Fe-P and Al-P (Durani and Tripathi 2017). Changes in soil P dynamics depend on the properties of soil and biochar (Bornø et al. 2018a; Mukherjee et al. 2019). Generally, the transformation of soil  $\text{P}_i$  fractions is affected by several factors such as the soil properties, soil development stage, P source type, rhizosphere processes (e.g., the effect of roots and microbial activity), and P uptake by plants (Negassa and Leinweber 2009; Wang et al. 2014; Zheng et al. 2002). When these factors are coupled with the differences in the raw materials and preparation temperatures of biochar, the effects of biochar on the content of various  $\text{P}_i$  fractions in the soil become inconsistent (Bornø et al. 2018a, b; Chathurika et al. 2016; Ch'ng et al. 2014a; Han et al. 2019; Wang et al. 2014).

Tobacco (*Nicotiana tabacum*) is one of the major economic crops grown worldwide (Lisuma et al. 2020). The total planting area of tobacco in China is 1.6 million hectares, accounting for 40% of the world's total output (Zhang et al. 2020). Tobacco solid waste, mainly consisting of the tobacco stem and discarded tobacco leaf, causes environmental pollution (Zheng et al. 2020). Furthermore, improper disposal may increase the risk of mosaic virus migration into the soil, eventually creating a negative impact on crop health in the following year (Zhang et al. 2020). Therefore, it is practically important to rationalize the treatment techniques for addressing tobacco waste. Biochar represents an alternative method of managing this waste that is more environmentally friendly (Zheng et al. 2020). Studies have shown that tobacco stalk biochar (TSB) application increases the organic matter content (15.98–293.13%), and the concentrations of available P (6.52–58.75%) and potassium (109.07–1789.70%) in tobacco-growing soil (Zheng et al. 2020). However, few studies have investigated the application of TSB to tobacco-growing soils, and it remains unclear whether the  $\text{P}_i$  fractions in different tobacco-growing soils consistently respond to biochar applications.

We hypothesized that TSB application would affect the soil  $\text{P}_i$  fractions and improve soil acidity. First, we predicted that the soil acidity will decrease with TSB application, and that the P content will be fixed by labile Fe and Al in the soil, thereby increasing the content of these fractions. Our second prediction was that TSB might affect the precipitation and dissolution balance of P, and the content of each fraction. To verify this hypothesis, we set up a field experiment and applied different amounts of TSB in two tobacco-planting areas in Bijie City, Guizhou Province, China, in 2018. The objectives were to (1) analyze the effect of biochar application on the changes in the  $\text{P}_i$  fractions and acidity in both the rhizosphere and bulk soils, (2) identify the factors influencing changes in the  $\text{P}_i$  fractions after biochar application, and (3) suggest an appropriate amount of biochar for enhancing the  $\text{P}_i$  fractions in tobacco fields.

## 2 Materials and Methods

### 2.1 Materials

The flue-cured tobacco variety used in this experiment was Yunyan 87. The biochar was made by the pyrolysis of tobacco stalks, under a limited supply of oxygen, at 380 °C for 2 h and was manufactured by the Guizhou Jinyefeng Agricultural Technology Corporation. The basic properties of the biochar were presented in Table S1.

## 2.2 Site Description and Experimental Design

The study areas are located in Bijie City, Guizhou Province, the main tobacco-planting area of China. The field experiments were conducted at two sites: Qianxi Linquan Science and Technology Demonstration Park (STDP), east of Bijie City, and Weining Heishi STDP in the west. Heishi, in Weining County (104.00° E, 26.76° N; 2120 m.a.s.l.), has a subtropical monsoon-humid climate, with an annual mean temperature and rainfall of 12.3 °C and 926 mm, respectively. This is in addition to an annual sunshine duration of 1066 h. The soil type is yellow-brown soil according to the Genetic Soil Classification of China (GSCC). Meanwhile, Linquan Town, in Qianxi County (106.04° E, 27.02° N; 1319 m.a.s.l.), has a subtropical warm and humid climate, with an annual mean temperature and rainfall of 14.2 °C and 1087 mm, respectively. The soil type is yellow soil according to the GSCC. The physical and chemical properties of the soils were provided in Table S2.

The field experiment began in May 2018 and included four biochar levels (0, 5, 20, and 40 t ha<sup>-1</sup>; labeled as B0, B5, B20, and B40, respectively) arranged in a randomized block design, with three replicates in each of the two sites. Each site had 12 plots, and the area of individual plots was 67 m<sup>2</sup> (8.7 m × 7.7 m) and 74.8 m<sup>2</sup> (11 m × 6.8 m) at Qianxi and Heishi, respectively. Biochar was evenly spread on the soil surface before ridging and then uniformly incorporated into the plow layer (0–20 cm depth) prior to the transplantation of the flue-cured tobacco.

## 2.3 Tobacco Planting and Field Management

The tobacco seedlings were transplanted in May 2018. Fertilization and field management were implemented following the local practices for high-quality tobacco. The fertilization scheme in the Qianxi site included 750 kg ha<sup>-1</sup> of distiller's grains organic fertilizer as the base fertilizer, with 37.5, 150, and 150 kg ha<sup>-1</sup> of compound fertilizer as topdressing (three times; the ratios of nitrogen, phosphorus, and potassium (N:P:K) were 22:6.1:8.3, 14:8.3:16.6, and 10.5:2.5:37.5, respectively). The fertilization scheme in the Heishi site had 1800 kg ha<sup>-1</sup> of distiller's grains organic fertilizer and 525 kg ha<sup>-1</sup> of compound fertilizer (N:P:K = 9:5.7:16.6) as the base fertilizer. This is in addition to 37.5 and 330 kg ha<sup>-1</sup> of compound fertilizer as topdressing (N:P:K = 15:3.5:5.8 and 13:0:21.6, respectively).

## 2.4 Soil Sampling

Soil samples were collected in August 2018 during the tobacco harvest period. Each bulk soil sample was a composite sample of the surface layer (0–20 cm) obtained with an auger (diameter 35 mm) from each plot using the five-spot sampling

method. At the same time, three tobacco plants were collected from each plot. The soil that was tightly attached to the root system of the tobacco plant was collected in a sealed plastic bag via the root-shaking method and thoroughly mixed as the rhizosphere soil. The soil samples were air-dried and sieved (2 mm) for measurements of the soil's chemical properties.

## 2.5 Measurement of Soil Chemical Properties

Soil pH of a 1:2.5 soil to water mixture was determined using a pH meter. Readily oxidized organic carbon (ROOC) was determined by colorimetry after 0.333 mol L<sup>-1</sup> potassium permanganate oxidation. Dissolved organic carbon (DOC) was extracted with deionized water and its concentration was determined using a TOC analyzer (Elementar Vario TOC, Germany). The levels of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N were determined using a digestion-continuous flow analyzer (AA3, SEAL, Germany) after extraction with 1 mol L<sup>-1</sup> KCl. The exchangeable and hydrolytic acidities were extracted with 1 mol L<sup>-1</sup> KCl and sodium acetate (NaOAc), respectively, and then titrated with a standard solution of 0.02 mol L<sup>-1</sup> sodium hydroxide (NaOH). Total N was determined using the Kjeldahl method and total P was measured with a sulfuric acid-perchloric acid system digestion-continuous flow analyzer (AA3, SEAL, Germany). Furthermore, Olsen-P was determined by molybdenum blue colorimetry after extraction with 0.5 mol L<sup>-1</sup> sodium bicarbonate (NaHCO<sub>3</sub>). Available potassium (AK) was extracted with 1 mol L<sup>-1</sup> ammonium acetate (NH<sub>4</sub>OAc) and analyzed by atomic absorption flame photometry (FP640, China). The soil organic carbon was measured via the potassium dichromate volumetric method. All these methods are detailed in the references of Bao (2000).

## 2.6 Soil P<sub>i</sub> Fractionation

Soil P<sub>i</sub> fractionation was performed by sequential extraction with different extractants following the method of Bao (2000). Briefly, Al-P, Fe-P, O-P, and Ca-P were extracted by 0.5 mol L<sup>-1</sup> ammonium fluoride (NH<sub>4</sub>F; pH 8.2), 0.1 mol L<sup>-1</sup> NaOH, 0.3 mol L<sup>-1</sup> sodium citrate and sodium hydrosulfite, and 0.5 mol L<sup>-1</sup> 1/2 H<sub>2</sub>SO<sub>4</sub>, respectively. The P concentrations in all of the extracts were analyzed using the molybdenum blue method. Details of this method can be found in the supplementary information section.

## 2.7 Statistical Analysis

The data were expressed as the means of three replicates on an oven-dry basis. Excel 2016 and SPSS 20.0 were used for the statistical analyses. One-way analysis of variance (ANOVA) was used to analyze the influence of the different biochar levels on the pH, exchangeable and hydrolytic acidities, and the concentrations of P<sub>i</sub> fractions in the soil. Duncan's

multiple range test was used to test significant differences among the treatments ( $P < 0.05$ ). Additionally, two-way ANOVA was used to determine the effects of the sites, treatments, and their interactions on the  $P_i$  fractions. Canoco 5.0 was used for redundancy analysis (RDA) of the  $P_i$  fractions and the chemical parameters.

### 3 Results

#### 3.1 Effects of Biochar on the Soil $P_i$ Fractions

##### 3.1.1 Soil Al-P Content

Biochar had varying effects on the Al-P content of the two experimental sites (Fig. 1). In Qianxi, the Al-P content in the bulk and rhizosphere soils was significantly higher in the B20 and B40 treatments than in the B0 and B5 treatments (Fig. 1a). However, in Heishi, the effects of biochar on the Al-P content of the bulk and rhizosphere soils were inconsistent. Overall, biochar significantly reduced the Al-P content of the rhizosphere soil, with no differences among the biochar application rates. Meanwhile, the Al-P content of the bulk soil was unaffected (Fig. 1b).

##### 3.1.2 Soil Fe-P Content

As shown in Fig. 2, the Fe-P content of the rhizosphere and bulk soils in Qianxi showed an initial increase, and then decreased with increasing biochar application rates. Only the B20 treatment at Qianxi had a significantly increased Fe-P concentration in the soil compared with B0. The Fe-P content in the bulk and rhizosphere soils was 350.60 and 499.42 mg kg<sup>-1</sup>, respectively (31.29–38.56% higher than that

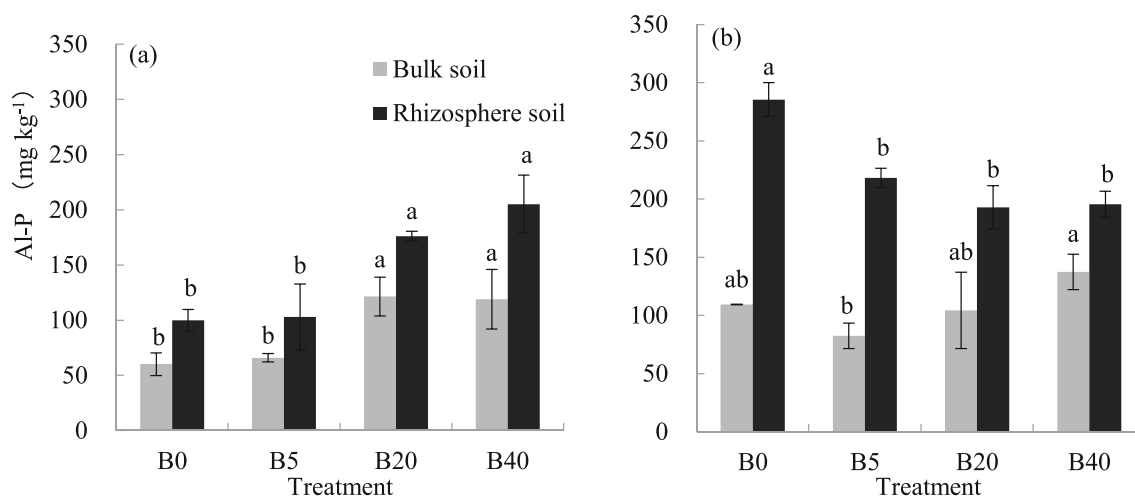
in B0; Fig. 2a). In Heishi, the Fe-P content in the bulk soil showed a tendency to decrease with increasing biochar application levels, but these differences were non-significant. In the rhizosphere soil, biochar application had negative effects on the Fe-P content in all treatments with the exception of B20 (Fig. 2b).

##### 3.1.3 Soil O-P Content

As shown in Fig. 3, biochar had varying effects on soil O-P in the two experimental sites. In Qianxi, while biochar did not affect the O-P content of the rhizosphere soil, it significantly ( $P < 0.05$ ) increased the O-P content in the bulk soil by 50.45–88.15% compared with the B0 treatment. Meanwhile, in the B20 treatment, the O-P content reached a maximum of 435.73 mg kg<sup>-1</sup>. In Heishi, biochar did not affect the O-P content of the bulk soil, but it significantly ( $P < 0.05$ ) reduced the O-P content in the rhizosphere soil (22.91–29.73% lower than that in B0). Nevertheless, no significant differences were detected among the biochar levels.

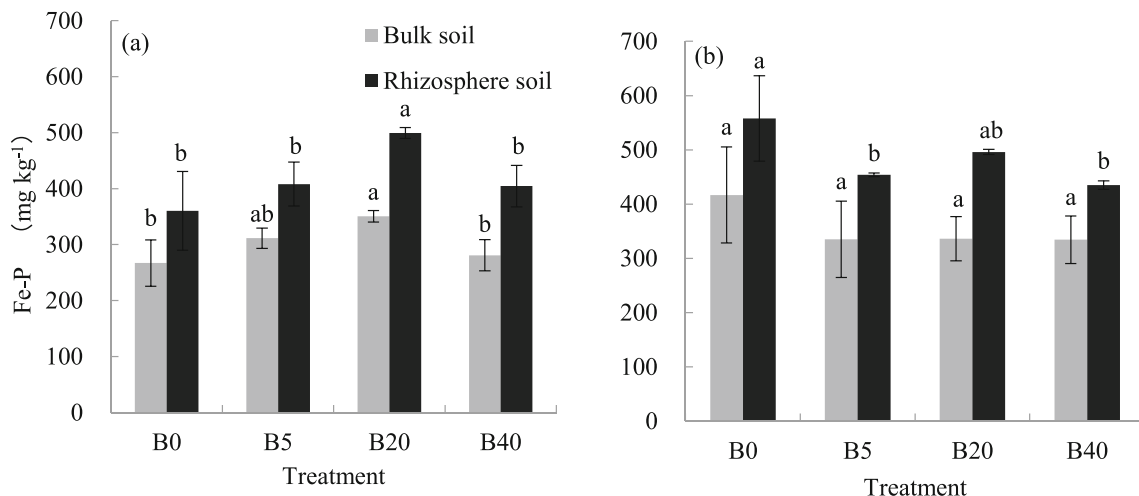
##### 3.1.4 Soil Ca-P Content

The soil Ca-P content in Heishi was higher than that in Qianxi, especially in the rhizosphere soil in Heishi, which 6.52 times higher Ca-P concentrations than that in Qianxi. In Qianxi, biochar application did not affect the Ca-P content of the bulk soil. However, in the rhizosphere soil, the B5 treatment significantly ( $P < 0.05$ ) reduced the Ca-P content (22.94 mg kg<sup>-1</sup>), while the B40 treatment significantly ( $P < 0.05$ ) increased the Ca-P content (76.01 mg kg<sup>-1</sup>). In Heishi, the Ca-P content of the soil was significantly reduced ( $P < 0.05$ ) in the biochar amendment plots when compared to the amendment plots without biochar (B0). Furthermore,



**Fig. 1** Effects of different biochar levels on aluminum-phosphate (Al-P) in the rhizosphere and bulk soils of two experimental sites: **a** Qianxi, **b** Heishi. B0, B5, B20, and B40 represent the application levels of tobacco stalk-derived biochar at 0, 5, 20, and 40 t ha<sup>-1</sup>, respectively. Different

lowercase letters represent significant differences among treatments by Duncan's test ( $P < 0.05$ ). The bar indicates the standard deviation of the triplicates



**Fig. 2** Effects of different biochar levels on iron-phosphate (Fe-P) in the rhizosphere and bulk soils of two experimental sites: **a** Qianxi, **b** Heishi. B0, B5, B20, and B40 represent the application levels of tobacco stalk-derived biochar at 0, 5, 20, and 40 t ha<sup>-1</sup>, respectively. Different

lowercase letters represent significant differences among treatments by Duncan’s test ( $P < 0.05$ ). The bar indicates the standard deviation of the triplicates

while there were no biochar-induced differences in the Ca-P content in the bulk soil, the Ca-P content in the rhizosphere soil of the B5 and B20 treatments was significantly reduced by 20.21% and 52.07%, respectively, compared with B0. Nevertheless, the difference between the results of the B40 and the B0 treatments was non-significant.

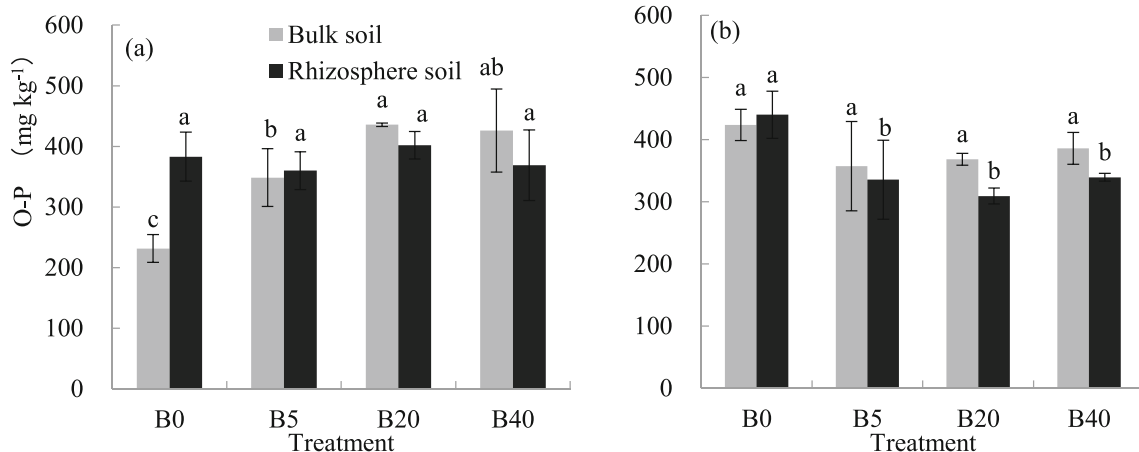
### 3.1.5 Effects on Soil P<sub>i</sub> Fractions Due to the Site, the Biochar Treatment, and Their Interactions

The two-way ANOVA results showed that the site had a significant ( $P < 0.05$ ) effect on all of the soil P<sub>i</sub> fractions, except soil O-P. Meanwhile, the biochar treatment had a significant ( $P < 0.05$ ) effect on all of the soil P<sub>i</sub> fractions, except Fe-P in the bulk soil (Table 1). The interaction between the sites and biochar applications also significantly ( $P < 0.05$ ) affected the

soil P<sub>i</sub> fractions, with the exception being Fe-P in the bulk soil. The Ca-P and Al-P content in the rhizosphere soil was especially affected; this was evidenced by an  $R^2$  value of 0.971 and 0.915, respectively.

### 3.2 Soil Acidity

As shown in Fig. S1, biochar did not affect the pH of the bulk soil in Qianxi ( $P > 0.05$ ), but it significantly ( $P < 0.05$ ) increased the pH of the bulk soil in Heishi, with the exception of the B5 treatment. Notably, larger amounts of biochar applications were associated with larger pH increases; for example, the pH of the B40 treatment increased by 1.47 units compared to the B0 treatment. However, biochar tended to lower the pH of the rhizosphere soil. The B20 treatment in Qianxi, and the



**Fig. 3** Effects of different biochar levels on occluded-phosphate (O-P) in the rhizosphere and bulk soils of two experimental sites: **a** Qianxi, **b** Heishi. B0, B5, B20, and B40 represent the application levels of tobacco stalk-derived biochar at 0, 5, 20, and 40 t ha<sup>-1</sup>, respectively. Different

lowercase letters represent significant differences among treatments by Duncan’s test ( $P < 0.05$ ). The bar indicates the standard deviation of the triplicates

**Table 1** Results (*P* values) of two-way analysis of variance (ANOVA) of site (S), treatment (T), and their interactions on inorganic phosphorus fractions of rhizosphere and bulk soils

P fraction	Al-P		Fe-P		Ca-P		O-P	
	<i>B</i>	<i>R</i>	<i>B</i>	<i>R</i>	<i>B</i>	<i>R</i>	<i>B</i>	<i>R</i>
S	0.036*	0.000**	0.018*	0.001*	0.000**	0.000**	0.193	0.176
T	0.000**	0.007*	0.557	0.025*	0.026*	0.000**	0.012*	0.041*
S×T	0.042*	0.000**	0.058	0.004*	0.000**	0.000**	0.000**	0.033*
<i>R</i> <sup>2</sup>	0.651	0.915	0.330	0.639	0.854	0.971	0.660	0.419

*B*, bulk soil; *R*, rhizosphere soil; *Al-P*, aluminum-phosphate; *Fe-P*, iron-phosphate; *Ca-P*, calcium-phosphate; *O-P*, occluded-phosphate

A single asterisk (\*) indicates a significant difference at  $P < 0.05$ . Double asterisk (\*\*) indicate a significant difference at  $P < 0.01$

B5 and B20 treatments in Heishi, had significantly ( $P < 0.05$ ) lower soil pH than that in the B0 treatment.

Except for the rhizosphere soil of the B5 treatment ( $0.06 \text{ cmol kg}^{-1}$ ), no exchangeable acidity was detected in the biochar treatments in Qianxi. Meanwhile, in Heishi, biochar significantly ( $P < 0.05$ ) reduced the exchangeable acidity of the rhizosphere and bulk soils. Furthermore, the hydrolytic acidity in the rhizosphere and bulk soils decreased with increasing biochar applications in both experimental sites.

Two-way ANOVA revealed that site and treatment had significant ( $P < 0.05$ ) effects on all of the soil acidities (Table 2). The interaction between site and treatment also significantly ( $P < 0.05$ ) affected the exchangeable acidity present in the bulk and rhizosphere soils ( $R^2 = 0.995$  and  $0.901$ , respectively), but it did not affect the hydrolytic acidity.

### 3.3 Relationship Between Soil $P_i$ Fractions and Soil Properties

Among the soil  $P_i$  fractions, only the soil Fe-P content had a significant positive correlation with hydrolytic acidity

( $P < 0.01$ ). There were no significant correlations between the other soil  $P_i$  fractions and soil acidity (Table 3).

The first and the second RDA axes explained 41.72% and 6.05% of the variance, respectively (Fig. 5). DOC, hydrolytic acidity, ROOC,  $\text{NO}_3^-$ -N, and AK were the important factors affecting the soil  $P_i$  fractions, which accounted for 19.3%, 8.8%, 6.5%, 6.2%, and 3.9% of the variance in the  $P_i$  fractions, respectively ( $P < 0.05$ ) (Table S3). Furthermore, the content of Al-P, Fe-P, and Ca-P in the soil was negatively correlated with the DOC content but positively correlated with the hydrolytic acidity (Fig. 5).

## 4 Discussion

### 4.1 The Influence of Biochar on Soil Fe-P and Al-P Content

The soil  $P_i$  fractions are usually Ca-P in alkaline soils, while in acidic soils, P normally combines with Fe, Al oxide, or hydroxide to form Fe-P, Al-P, and O-P (Ngatia et al. 2017). In

**Table 2** Effects of different application levels of biochar on exchangeable and hydrolytic acidities in the rhizosphere and bulk soils of two experimental sites

Treatment	Exchangeable acidity( $\text{cmol kg}^{-1}$ )				Hydrolytic acidity( $\text{cmol kg}^{-1}$ )			
	Qianxi		Heishi		Qianxi		Heishi	
	<i>B</i>	<i>R</i>	<i>B</i>	<i>R</i>	<i>B</i>	<i>R</i>	<i>B</i>	<i>R</i>
B0	0.00 <sup>a</sup>	0.00 <sup>b</sup>	0.56 <sup>a</sup>	0.31 <sup>a</sup>	4.74 <sup>a</sup>	6.23 <sup>a</sup>	6.96 <sup>a</sup>	7.59 <sup>a</sup>
B5	0.00 <sup>a</sup>	0.06 <sup>a</sup>	0.14 <sup>b</sup>	0.33 <sup>a</sup>	4.26 <sup>a</sup>	5.46 <sup>ab</sup>	6.28 <sup>a</sup>	7.18 <sup>ab</sup>
B20	0.00 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	0.02 <sup>b</sup>	3.47 <sup>ab</sup>	4.38 <sup>ab</sup>	3.89 <sup>b</sup>	5.65 <sup>bc</sup>
B40	0.00 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>	0.00 <sup>b</sup>	1.62 <sup>b</sup>	3.74 <sup>b</sup>	1.87 <sup>c</sup>	4.81 <sup>c</sup>
	<i>B</i>		<i>R</i>		<i>B</i>		<i>R</i>	
Site (S)	0.000**		0.000**		0.000**		0.002*	
Treatment (T)	0.000**		0.000**		0.002**		0.006*	
S×T	0.000**		0.000**		0.111		0.959	
<i>R</i> <sup>2</sup>	0.995		0.901		0.816		0.554	

*B*, bulk soil; *R*, rhizosphere soil. B0, B5, B20, and B40 represent application biochar levels at 0, 5, 20, and 40 t  $\text{ha}^{-1}$

Different lowercase letters in the same column indicate a significant difference at  $P < 0.05$

**Table 3** Correlation coefficient between soil inorganic phosphorus fractions and soil acidity

P fractions	pH	Exchangeable acidity	Hydrolytic acidity
Al-P	0.222	0.077	0.238
Fe-P	-0.090	0.296	0.641**
O-P	0.021	0.251	0.159
Ca-P	-0.085	0.347	0.473

Al-P, aluminum-phosphate; Fe-P, iron-phosphate; Ca-P, calcium-phosphate; O-P, occluded-phosphate

Double asterisks (\*\*) indicate significant correlation at the 0.01 level (both sides)

the present study, the soils are acidic (pH 5.3–5.4) and the content of soil  $P_i$  fractions in the two experimental sites are as follows: Fe-P > O-P > Al-P > Ca-P (Table S2). This is mainly attributed to the high Fe and Al content in acidic soil. Notably, Al-P and Fe-P are also effective fractions for crops in acidic soils (Ch'ng et al. 2014a, b). Biochar is a special soil conditioner with high P levels that can directly affect the  $P_i$  fractions in the soil. However, biochar incorporated into the soil will also change the pH, and the physical and chemical properties of the soil, which indirectly affect soil P concentrations (Gao and DeLuca 2020; Zhou et al. 2020). Mukherjee et al. (2019) reported that rice residual biochar directly increased soil P availability by releasing P from biochar itself, or indirectly by either reducing P sorption or mobilizing P. The content of total P (2.38 g kg<sup>-1</sup>) and Olsen-P (822.44 mg kg<sup>-1</sup>) in the TSB used in this study (Table S1) was much higher than that in the test soil (Table S2). Biochar application at 5–40 t ha<sup>-1</sup> was equivalent to the addition of 11.9–95.2 kg ha<sup>-1</sup> of total P and 4.11–32.90 kg ha<sup>-1</sup> of Olsen-P into the soil, which directly increased the soil P content and affected the  $P_i$  fractions. Orthophosphate and pyrophosphate are the main P species in manure biochar, which can directly increase the content of soil  $P_i$ . In addition, biochar enhanced the activity of alkaline phosphomonoesterase, which can decompose certain organic P such as monoesters (Jin et al. 2016). Overall, biochar application increased the content of soil Al-P and Fe-P in the Qianxi experimental site. However, in the Heishi site, the Al-P and Fe-P levels underwent reduction and had no change, (Figs. 1, 2). These differences could be attributed to the different soil types, climate conditions, and the amount of base fertilizers used in the two experimental sites. Bornø et al. (2018b) utilized three biochar types from different feedstocks, with or without P fertilizers, and found no effect on the content of NaOH-extractable  $P_i$  (NaOH- $P_i$ , e.g., Fe-P and Al-P) in the bulk soil. However, the NaOH- $P_i$  fraction in rhizosphere soil increased significantly ( $P < 0.01$ ) after treatment with biochar that had no P fertilizer. In addition, Mukherjee et al. (2020) reported that the content of Al-P and Fe-P increased significantly with the increase in biochar

application, regardless of the initial total P status of the test soil. Furthermore, Wang et al. (2014) found that biochar promoted increases in the soil resin-extractable P (resin-P) and NaOH- $P_i$ , and the sum of their increased quantity was almost equal to the amount of P added. This indicated a total recovery of biochar P by resin and NaOH extraction. Ch'ng et al. (2014a, b) showed that the content of Al-P and Fe-P in the soil increased after biochar addition, while Hong and Lu (2018) showed that biochar could significantly increase Al-P content and decrease Fe-P content. These studies demonstrated that biochar could affect the content of Al-P and Fe-P in the soil. However, these effects depended on the type and amount of biochar, as well as the soil type. Moreover, the amount base fertilizer in Heishi (1800 kg ha<sup>-1</sup> of organic fertilizer together with 525 kg ha<sup>-1</sup> of chemical fertilizer) was approximately three times higher than that in Qianxi (750 kg ha<sup>-1</sup> of organic fertilizer). Therefore, the P content was higher in Heishi owing to the Fe-P and Al-P content in B0 treatment.

There are three main forms of P fixation in acidic soils. The first is simple precipitation to form iron (III) phosphate (FePO<sub>4</sub>; solubility product constant ( $K_{sp}$ ) =  $1.3 \times 10^{-22}$ ) and aluminum-phosphate (AlPO<sub>4</sub>;  $K_{sp}$  =  $5.8 \times 10^{-19}$ ). The second is the formation of strengite (Fe(OH)<sub>2</sub>·H<sub>2</sub>PO<sub>4</sub>,  $pK_{sp}$  = 33–35) or phosphoaluminite (Al(OH)<sub>2</sub>·H<sub>2</sub>PO<sub>4</sub>,  $K_{sp}$  =  $3.15 \times 10^{-30}$ ), mainly due to the large amounts of Fe<sup>3+</sup> and Al<sup>3+</sup> in strongly acidic soils and the H<sub>2</sub>PO<sub>4</sub><sup>-</sup> (>10<sup>-6</sup> mol L<sup>-1</sup>) in the soil solution or P fertilizer that is easily fixed. The third is the amorphous colloid, Fe(OH)<sub>3</sub>, which can form a Fe(OH)<sub>3</sub> film on the surface of strengite in the soil to form O-P. The solubility of Fe(OH)<sub>3</sub> ( $K_{sp}$  =  $4 \times 10^{-38}$ ) is much smaller than that of strengite, which makes it less effective than strengite as a source of P. Furthermore, the P supplied by P fertilizer and biochar will be quickly fixed by free Fe<sup>3+</sup> and Al<sup>3+</sup> in acidic soils, thereby decreasing their availability. Conversely, biochar may occupy the P adsorption site, causing P to be fixed by Fe<sup>3+</sup> and Al<sup>3+</sup> after its release. The pH value of soil increased after biochar application, which resulted in the dissolution of phosphates bounding to the free cations (e.g., Fe<sup>3+</sup>, Al<sup>3+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>), thereby releasing available P for the plant (Hong and Lu 2018).

Soil pH is the negative logarithm of hydrogen ion (H<sup>+</sup>) activity in soil solutions, which is the active acidity of soil and a strong factor. Soil acidity mainly depends on the amount of potential acidity, especially hydrolytic acidity, which is the capacity index of soil acidity. Studies have shown that biochar application increases the soil pH and decreases the exchangeable acidity and exchangeable Al content (Kamran et al. 2018; Masulili et al. 2010; Mehmood et al. 2017). In the present study, the lowering of pH of the rhizosphere soil after biochar application may be associated with biochar-induced improvements to soil properties, such as the water retention characteristics (Hussain et al. 2020), nutrient availability (Luo et al. 2020), and the act of roots secreting more organic acids (Pei

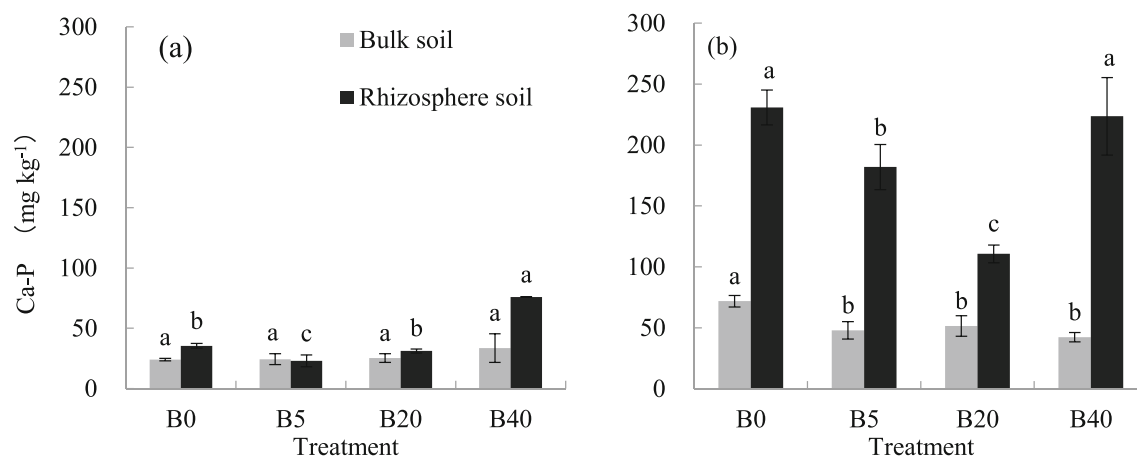
et al. 2020). In the present study, biochar significantly reduced the content of exchangeable and hydrolytic acids found in soil (Table 3). This could be attributed to the soluble exchangeable cations such as  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  in the biochar, which can increase the soil cation saturation, and reduce the content of  $\text{H}^+$  and exchangeable  $\text{Al}^{3+}$  in the soil. Furthermore, the neutralization of alkaline substances on the surface of biochar was found to reduce soil acidity. Biochar increased the soil pH, and as the concentration of  $\text{OH}^-$  in the soil increased, strengite and phosphorite began to dissolve, leading to P release. Increased  $\text{OH}^-$  in the soil can also influence the exchange reaction of  $\text{H}_2\text{PO}_4^-$  and  $\text{OH}^-$  on the surface of soil minerals, causing the reaction to reverse and re-release  $\text{H}_2\text{PO}_4^-$ . In this case, the P fixed by Fe and Al can still become available P. Moreover, the high P content of biochar may reduce the exchangeable Al content in the soil, via precipitation with Al. This can also reduce the exchangeable acidity of the soil (Garrido et al. 2003; Illera et al. 2004) and increase the Al-P content. Wan et al. (2014) reported that the beneficial effects of biochar in acidic soils increased with increasing pyrolysis temperatures. In summary, the effects of biochar on soil pH are closely related to the soil conditions, soil microenvironment, biochar type, biochar pyrolysis temperature, and biochar amount.

Changes in the Al-P and Fe-P content in the soil after biochar addition may be related to the comprehensive effects of the above processes. As indicated by Xu et al. (2013), P solubility with biochar application is complicated because it is affected by soil pH, Fe and Al oxides, and direct P contributions from biochar. Moreover, Fe and Al oxides are more complexly affected by the soil pH and sorption due to biochar. After biochar application, when the soil content of Al-P and Fe-P is low, the main reaction is the fixation of P. However, when higher Al-P and Fe-P content is present, the comprehensive reactions depend on the dissolution of P and eventually

reach an equilibrium state. In this study, the equilibrium amounts of Al-P and Fe-P were approximately  $200 \text{ mg kg}^{-1}$  and  $500 \text{ mg kg}^{-1}$ , respectively.

#### 4.2 The Influence of Biochar on Soil O-P and Ca-P Content

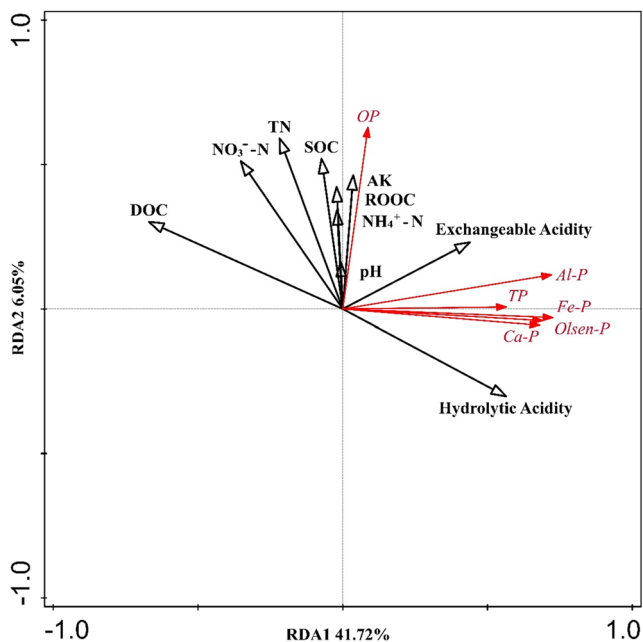
O-P is a phosphate coated with an Fe oxide film (or another film). The solubility of Fe oxide is very low; therefore, the P in this fraction is rarely absorbed by plants. In this study, biochar did not affect the O-P in the Qianxi rhizosphere soil or the Heishi bulk soil, while it increased the O-P content in the Qianxi bulk soil (Fig. 3). Cao et al. (2020a) reported that in both bulk and rhizosphere soils, P fractions were affected by biochar. They also noted that the degree of this effect was different between bulk soil and rhizosphere soil. Xu et al. (2016) showed that water-soluble or organic P was transformed into a labile or semi-labile pool, and eventually into a stable pool, during the pyrolysis process. They went on to report that more stable P was formed at higher pyrolysis temperatures. Therefore, biochar application may contribute a portion of stable P into soil. Additionally, biochar may have promoted the formation of the film in the Qianxi bulk soil and significantly increased the O-P content. Conversely, the O-P content of the Heishi rhizosphere soil was significantly reduced, and this phenomenon may be related to a reduction in the rhizosphere redox potential that caused O-P to dissolve and become unbound. Chaturika et al. (2016) found that biochar did not affect the residual P (O-P) content of two different soils during a 70-day experiment, but other studies have shown that biochar significantly reduced or did not affect the O-P content (Cao et al. 2020a; Cao et al. 2020b; Wei et al. 2020). In those cases, O-P is thought to transform into dicalcium phosphate ( $\text{Ca}_2\text{-P}$ ), Fe-P, and octacalcium phosphate ( $\text{Ca}_8\text{-P}$ ) forms.



**Fig. 4** Effects of different biochar levels on calcium-phosphate (Ca-P) in the rhizosphere and bulk soils of two experimental sites: **a** Qianxi, **b** Heishi. B0, B5, B20, and B40 represent the application levels of tobacco stalk-derived biochar at 0, 5, 20, and 40  $\text{t ha}^{-1}$ , respectively. Different

lowercase letters represent significant differences among treatments by Duncan's test ( $P < 0.05$ ). The bar indicates the standard deviation of the triplicates





**Fig. 5** Effects of soil chemical properties on soil inorganic phosphorus fractions based on the redundancy analysis (RDA). DOC, dissolved organic carbon; TN, total nitrogen; ROOC, readily oxidized organic carbon; SOC, soil organic carbon; AK, available potassium; Al-P, aluminum-phosphate; Fe-P, iron-phosphate; O-P, occluded-phosphate; Ca-P, calcium-phosphate

In this study, the effects of biochar on Ca-P were inconsistent (Fig. 4). Mahmoud et al. (2019) found that the soil Ca-P content decreased after biochar application under normal and 150% of the normal P application rates, but did not change under less than normal P fertilizer application. Bornø et al. (2018b) also reported pronounced variability in the Ca-P fractions; however, a significant Ca-P increase was observed in rice husk biochar-treated rhizosphere soil. Furthermore, Gerdelidani and Hosseini (2018) found that biochar only increased Ca<sub>2</sub>-P significantly in sandy loam soil, one of the three calcareous soils with different textures used in their study. Additionally, Mukherjee et al. (2020) showed a significant increase in Ca-P content with increasing rate of biochar application. The result is consistent with that of Ch'ng et al. (2014a, b) who also observed that amending soil with biochar increased the soil Ca-P fraction. The increase of Ca-P fraction could be related to the chemistry and retention of Ca. To determine this, Xu et al. (2018) used a modified Hedley method to study the P transformation in the soil. They found that biochar had a positive effect on most forms of P in Haplic Luvisol, but negative effects were observed on various forms of P in the Calcaric-Fluvisol.

Overall, the effects of biochar on soil P<sub>i</sub> fractions are inconsistent, which may be due to the soil properties, soil development stage, types of P sources, rhizosphere processes (e.g., the effect of root and microbial activities), plant uptake of P, and management practices (Negassa and Leinweber 2009; Wang et al. 2014; Zheng et al. 2002).

## 5 Conclusion

In this study, we investigated the effects of tobacco stalk biochar (TSB) on inorganic phosphorus (P) fractions in tobacco-growing soil. Our findings showed that the application of TSB affected the soil inorganic P (P<sub>i</sub>) fractions through ligand exchange, precipitation, and dissolution reactions. The high P content and exchangeable cations contained within the biochar itself also had roles to play in affecting P<sub>i</sub> fractions. Furthermore, we found that the change in soil P<sub>i</sub> fractions was influenced by the amount of biochar applied, the experimental site, and the tobacco roots. Redundancy analysis indicated that dissolved organic carbon (DOC), hydrolytic acidity, readily oxidized organic carbon (ROOC), NO<sub>3</sub><sup>-</sup>-N, and available potassium (AK) were the important factors affecting the soil P<sub>i</sub> fractions. Furthermore, we found that the content of the soil P<sub>i</sub> fractions negatively correlated with the DOC content, but positively correlated with the hydrolytic acidity. Overall, the results suggest that the appropriate amount of TSB to be used for enhancing soil P<sub>i</sub> fractions in our field experiment is 20 t ha<sup>-1</sup>. However, future studies are necessary to validate these findings through several field trials in diverse soils.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s42729-021-00472-6>.

**Funding** This work was financially supported by the key project of the China Tobacco Corporation (“Study on the Mechanism and Regulation Technology of Organic Carbon Function Based on the Balance of Carbon and Nitrogen in Tobacco Field”; 110,201,902,004). The science and technology project of the Bijie Company of Guizhou Tobacco Company (“Mechanism of Soil Fertility Conservation and Nutrient Bio-efficiency of Biochar in Tobacco Field”; 2018520500240065) also contributed financially to this study.

## Declarations

**Conflict of Interest** The authors declare that they have no conflict of interest.

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