Effects of Biochar Amendment on Soil Phosphorus Fractions and Transformations in Eucalyptus Plantations (Postprint)

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Abstract

To ameliorate the widespread phosphorus (P) deficiency in soils of subtropical eucalyptus plantations in China, this study utilized biochar (BC) as a soil amendment, with soil from a 15-year-old eucalyptus plantation as the research subject. Through a laboratory incubation experiment, different proportions of BC were added at rates of 0 (CK), 2%, 5%, 10%, and 20% to investigate the effects of different BC application rates on soil P fractions and transformation and their relationships with soil physicochemical properties. The results showed that: (1) Compared with CK, the 20% BC addition rate significantly increased soil ammonium nitrogen (NO3–N), total phosphorus (TP), microbial biomass phosphorus (MBP) content, and pH value ($P<0.05$), while the 2%, 5%, and 10% BC addition rates only significantly increased MBP and pH value ($P<0.05$), with no significant effects on other soil physicochemical indicators. (2) Compared with CK, the 2% BC addition rate significantly increased labile P (LP) ($P<0.05$); the 5% and 10% BC addition rates significantly increased both very labile P (AP) and LP ($P<0.05$); and the 20% BC addition rate significantly increased AP, LP, and occluded P (OP) ($P<0.05$), while moderately labile P (MP) showed no significant changes under the four BC addition rates. (3) The activities of β-glucosidase (BG), N-acetylglucosaminidase (NAG), protease (LAP), and acid phosphatase (ACP), which are related to C, N, and P transformations, were all significantly higher than CK under the 10% and 20% BC addition rates ($P<0.05$). (4) Correlation analysis results showed that both ln(BG) and ln(NAG+LAP) were significantly positively correlated with ln(ACP) ($P<0.05$); redundancy analysis (RDA) indicated that pH, TN, and TP were the most important factors driving changes in soil P fractions in eucalyptus plantations, while structural equation model (SEM) further demonstrated that pH, C:P, and N:P were the key factors driving soil P transformation. In summary, different BC application rates primarily improved enzyme activities related to C and N cycling by influencing soil physicochemical properties, and consequently enhanced the P supply potential of eucalyptus plantation soils to some extent, with the high BC addition rate (20%) showing the best effect. This study holds important significance for guiding soil nutrient management in eucalyptus plantations and promoting sustainable forestry development in China.

Full Text

Effects of Biochar Addition on Soil Phosphorus Composition and Transformation in Eucalyptus Plantations

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Abstract

To address the widespread phosphorus (P) deficiency in subtropical eucalyptus plantation soils in China, this study investigated the effects of biochar (BC) as a soil amendment on soil P fractions and transformation. Using soil from a 15-year-old eucalyptus plantation, we conducted a laboratory incubation experiment with different BC application rates [0 (CK), 2%, 5%, 10%, and 20%] to examine how varying BC amounts influence soil P composition and transformation and their relationships with soil physicochemical properties. The results showed: (1) Compared to CK, the 20% BC addition rate significantly increased soil nitrate nitrogen (NO_3^--N), total phosphorus (TP), microbial biomass phosphorus (MBP) content, and pH (P<0.05), while 2%, 5%, and 10% BC addition rates only significantly increased MBP and pH (P<0.05) without significantly affecting other soil physicochemical indicators. (2) Compared to CK, the 2% BC addition rate significantly increased labile P (LP) (P<0.05); the 5% and 10% rates significantly increased both readily available P (AP) and LP (P<0.05); and the 20% rate significantly increased AP, LP, and occluded P (OP) (P<0.05), while moderately available P (MP) showed no significant changes across all four BC addition rates. (3) The activities of β-glucosidase (BG), N-acetyl-glucosaminidase (NAG), protease (LAP), and acid phosphatase (ACP)—enzymes related to C, N, and P transformation—were all significantly higher at 10% and 20% BC addition rates compared to CK (P<0.05). (4) Correlation analysis revealed that ln(BG) and ln(NAG+LAP) were both significantly positively correlated with ln(ACP) (P<0.05). Redundancy analysis (RDA) indicated that pH, TN, and TP were the primary factors driving changes in soil P fractions in eucalyptus plantations, while structural equation modeling (SEM) further demonstrated that pH, C:P, and N:P ratios were the most critical factors driving soil P transformation. In conclusion, different BC application rates primarily improved enzyme activities related to C and N cycling by influencing soil physicochemical properties, thereby enhancing the P supply potential of eucalyptus plantation soils to varying degrees, with the highest concentration (20%) showing the optimal effect. This study provides important guidance for soil nutrient management and sustainable forestry development in China's eucalyptus plantations.

Keywords: biochar, eucalyptus plantation, soil phosphorus fraction, soil enzyme activity, subtropics

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Introduction

Phosphorus (P) is an essential mineral nutrient element throughout the plant life cycle (Cao et al., 2014), participating in the synthesis of sugars, proteins, chlorophyll, and other substances, and involved in all physiological and biochemical processes from individual development to natural death. Soil P is the source for plant P acquisition, and its form and structure directly affect soil P bioavailability (Li et al., 2015). Soil P exists in two forms: inorganic phosphorus (Pi) and organic phosphorus (Po) (Gao et al., 2022). Pi mostly occurs as orthophosphate, which in southern China—dominated by eroded red acidic soils—often combines with Fe and Al to form phosphates that are difficult for plants to absorb. Po is abundant in global soil P pools, accounting for approximately 15%–80% of total P, but must undergo biological mineralization to become soluble phosphate before plant uptake (Achat et al., 2009). Due to the rainy climate conditions in subtropical regions, available P in soils experiences "leaching" and is lost in large quantities with percolating water, resulting in insufficient soil P supply (Zhang et al., 2021). Consequently, P has long been considered one of the most critical factors limiting productivity and sustainable management of plantation ecosystems in this region (Crous et al., 2015). In-depth study of P fraction changes and transformation mechanisms in subtropical plantation soils can help improve soil P effectiveness and maintain ecosystem productivity and sustainable development of ecological processes.

Biochar (BC) is the product of biomass pyrolysis under anoxic or hypoxic conditions (Liu et al., 2019). Research shows that BC, as a novel material, can effectively improve soil and enhance soil fertility after artificial input into soil ecosystems (Gao et al., 2021). The porosity of BC benefits soil porosity increase and bulk density reduction (Zhao et al., 2021), while its large specific surface area can release substantial charges, effectively adsorbing N, P, and other elements to promote soil nutrient retention (Wu et al., 2014). Studies indicate that BC maintains strong capacity for sustaining soil productivity and fertility even at low application rates (Schulz et al., 2013). Additionally, BC's adsorption of cations such as Ca^{2+}, Fe^{3+}, and Al^{3+} can reduce cation-P binding, increasing available P content in soil (Liu et al., 2017). Xu et al. (2014) evaluated four BC application rates (0, 1%, 5%, and 10%, w/w) on soil P and found that BC application could alter soil P adsorption and desorption capacity, thereby changing soil P availability. BC addition not only directly increases available P content but also modifies soil physicochemical properties and microbial communities, subsequently affecting soil P adsorption and mineralization (Kloss et al., 2014). Pandit et al. (2018) found that soil pH increased with BC addition, enhancing negative charges on soil mineral surfaces, reducing P adsorption, and increasing soil P availability. Furthermore, BC can influence microbial activity and promote P mineralization in soil (Li et al., 2019), possibly because: (1) active functional groups on BC surfaces enhance interactions with soil, creating suitable habitats for microorganisms involved in soil nutrient cycling (Zhao et al., 2021); and (2) BC can increase soil organic carbon content, thereby enhancing soil microbial activity (Demisie et al., 2014). Soil enzyme activity is an important indicator for evaluating soil quality and microbial activity (Liu et al., 2017). Therefore, investigating the effects of different BC application rates on soil nutrients and enzyme activities to analyze P fraction changes and transformation processes is crucial for improving BC application methods, increasing soil available P content, reducing P loss, enhancing soil quality, and maintaining plantation ecosystem stability.

Eucalyptus, characterized by fast growth, multiple uses, high economic value, and wide adaptability, has been extensively planted in southern China, making important contributions to forestry production and economic development (Wang et al., 2021). However, extensive planting and continuous multi-generation cultivation of eucalyptus plantations can easily lead to soil compaction, poor aeration, and rapid fertility decline, seriously affecting soil quality and sustainable plantation management (Wen et al., 2019). Costa et al. (2016) found that available P pools are affected by Pi dissolution and Po mineralization, with available P deficiency occurring in P-deficient eucalyptus forest sites or under low P fertilizer supply conditions. Although eucalyptus plantations can significantly increase growth rates through P fertilizer addition (Valadares et al., 2020), excessive P fertilizer application causes soil P surplus, which enters rivers and lakes with rainfall or surface runoff, wasting resources and causing environmental pollution (Ji et al., 2021). While reports exist on BC application altering soil P availability (Xu et al., 2014), the effects of BC on P form transformation in subtropical eucalyptus plantation soils and its regulatory mechanisms remain poorly understood (Foltran et al., 2019), and specific interactions between BC application rates and soil P transformation have been relatively understudied, greatly limiting BC application and promotion in this region. Therefore, this study used subtropical long-term managed eucalyptus plantation soil (red soil) as the research object, employing laboratory incubation experiments to investigate the effects of different BC application rates on soil P fraction changes, and to elucidate the main pathways and key driving factors of BC-regulated soil P transformation, providing scientific guidance for selecting appropriate BC application rates to improve soil P availability in eucalyptus plantations.

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1. Materials and Methods

1.1 Experimental Materials and Design The study area is located in Shaoping Forest Farm, Tropical Forestry Experimental Center, Chinese Academy of Forestry, in Pingxiang City, Guangxi Zhuang Autonomous Region, China (106°51 –106°53 E, 22°02 –22°04 N). The region has a typical subtropical monsoon climate with abundant sunlight, mean annual temperature of 20.5–21.7°C, and concentrated rainfall from April to September with mean annual precipitation of approximately 1,401 mm. The main landform is low hills, and the soil type is acidic red soil formed from granite weathering under high temperature and alternating wet-dry conditions.

Based on the principle that P in BC has more stable thermochemical properties when carbonization temperature exceeds 600°C (Johan et al., 2021), this experiment selected biochar produced from rice straw at 600°C as the amendment material. Surface soil (0–10 cm) was randomly collected from a 15-year-old eucalyptus plantation as the test soil. The basic physicochemical properties of BC and test soil are detailed in Table 1 . The collected soil (~30 kg) was air-dried, passed through a 2 mm sieve, and bagged for later use. A laboratory incubation experiment was conducted with five BC addition treatments: 0 (CK), 2% (2B), 5% (5B), 10% (10B), and 20% (20B), with five replicates per treatment. BC and soil were thoroughly mixed, placed in incubation bottles (125 ml capacity with ventilated caps), and compacted to approximate field bulk density. Samples were incubated in darkness at constant temperature (25°C), with water added periodically to maintain 40% of maximum water-holding capacity. After one year of incubation, soil physicochemical properties, P fractions, and enzyme activities were measured.

1.2 Experimental Methods Soil bulk density and maximum water-holding capacity were measured using the ring knife method (Liu, 1996). Soil pH was determined at a soil-to-water mass ratio of 1:2.5. Soil total organic carbon (SOC) was measured using the potassium dichromate-sulfuric acid external heating method (Nelson et al., 1982). Total nitrogen (TN) was determined by the Kjeldahl method. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were extracted with 50 mL of $CaCl_2$ (0.01 $mol \cdot L^{-1}$) solution added to fresh soil equivalent to 10 g dry weight, and measured using a continuous flow analyzer (Huang et al., 2014). Total phosphorus (TP) was digested with $HClO_4$-H_2SO_4 (Vance et al., 1987) and determined by the molybdenum-antimony anticolorimetric method (Murphy & Riley, 1962).

Soil microbial biomass phosphorus (MBP) was measured using the chloroform-fumigation extraction method (Vance et al., 1987). Three 5 g soil samples were prepared: the first two for fumigated and non-fumigated comparison, and the third non-fumigated sample with 250 µL KH_2PO_4 (250 $mol \cdot mL^{-1}$) added. All three samples were extracted with 40 mL $NaHCO_3$ (0.5 $mol \cdot mL^{-1}$) solution and determined by the molybdenum-antimony anti-colorimetric method (Murphy & Riley, 1962).

Soil enzyme activities involved in C, N, and P cycling were determined using fluorometric microplate assays (Saiya-Cork et al., 2002). Fresh soil equivalent to 1.25 g dry weight was added to 125 mL ultrapure water and homogenized for 55 s at 4°C using a micro-blender to create a soil suspension. Each sample had eight replicates. After adding enzyme substrates, samples were incubated in darkness at 25°C for 4 h, then measured using a microplate reader (wavelength 365–450 nm). All enzyme activities were uniformly expressed in $nmol \cdot h^{-1} \cdot g^{-1}$ soil. Enzyme types, functions, and substrate information are detailed in Table 2 .

Soil P fractionation was performed using the Hedley method (Zhang et al., 2009), which simultaneously considers Pi and Po fractions including Resin-P, $NaHCO_3$-P, microbial cell P (Microbial-P), NaOH-soluble P (NaOH-P), intra-aggregate P (ultrasonic dispersion/NaOHs-P), apatite P (HCl-P), and residual P (Residual-P). All seven P fractions were determined by the molybdenum-antimony anti-colorimetric method (Demisie et al., 2014). These fractions were categorized into four groups based on plant and microbial availability: readily available P (AP), labile P (LP), moderately available P (MP), and occluded P (OP) (Yang & Post, 2011). Resin-P was classified as AP; $NaHCO_3$-P and Microbial-P as LP; NaOH-P as MP; and NaOHs-P, HCl-P, and Residual-P as OP.

1.3 Data Analysis Statistical analyses were performed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to analyze differences in soil physicochemical properties, MBP, soil P fractions, and enzyme activities among different BC addition rates, with least significant difference (LSD) tests for mean comparisons at $P<0.05$ significance level. Pearson correlation analysis was used to examine relationships between enzyme stoichiometries. Principal components analysis (PCA) was used to differentiate changes in soil P fractions after different BC additions, and redundancy analysis (RDA) was used to identify main factors influencing P fraction changes. PCA and RDA were performed using Canoco 5.0 (Biometris-Plant Research International, Wageningen, The Netherlands). Structural equation modeling (SEM) was used to construct regulatory pathways of BC addition affecting ACP transformation, analyzed using Amos 24.0 (SPSS Inc., Chicago, IL). All figures were created using Sigma Plot 12.0 and Microsoft Office Visio 2007.

2. Results

2.1 Effects of Different Biochar Application Rates on Soil Physicochemical Properties Different BC application rates caused varying degrees of change in soil pH, SOC, TN, NH_4^+-N, NO_3^--N, TP, and MBP in eucalyptus plantations. As shown in Table 3 , compared to CK, soil pH significantly increased by 10.1%, 20.8%, 24.8%, and 40.1% under the four addition rates (2%, 5%, 10%, and 20%), respectively (P<0.05). At the 20% addition rate, NO_3^--N and TP significantly increased by 51.6% and 110.7%, respectively (P<0.05). MBP significantly increased by 149.7%, 258.7%, 205.4%, and 350.5% under the four BC addition rates, respectively (P<0.05). In contrast, SOC and TN showed no significant changes under any of the four BC addition rates compared to CK.

2.2 Effects of Different Biochar Application Rates on Soil P Fractions Compared to CK, AP increased after BC addition, reaching significant levels at 10% and 20% addition rates with increases of 10.0% and 24.9%, respectively (Figure 1 [Figure 1: see original paper]A). LP significantly increased by 1.6%, 5.4%, 14.9%, and 22.4% at 2%, 5%, 10%, and 20% addition rates, respectively (Figure 1B) (P<0.05). MP showed no significant effects across the four BC addition rates (Figure 1C). Additionally, OP significantly increased by 42.5% at the 20% addition rate (Figure 1D) (P<0.05).

2.3 Effects of Different Biochar Application Rates on Soil Enzyme Activities and Enzyme Stoichiometry Compared to CK, BG activity significantly increased by 63.8%, 68.0%, 80.8%, and 131.8% under the four addition rates, respectively (Figure 2 [Figure 2: see original paper]A) (P<0.05). NAG significantly increased by 14.1%, 15.3%, and 30.6% at 2%, 10%, and 20% addition rates, respectively (Figure 2B) (P<0.05). LAP significantly increased by 160.0%, 90.3%, and 231.0% at 5%, 10%, and 20% addition rates, respectively (Figure 2C) (P<0.05). ACP significantly increased by 17.2% and 44.4% at 10% and 20% addition rates, respectively (Figure 2D) (P<0.05).

As shown in Figure 3 [Figure 3: see original paper], both ln(BG) and ln(NAG+LAP) showed significant positive linear correlations with ln(ACP) (P<0.05).

2.4 Effects of Different Biochar Application Rates on Soil P Fractions and Transformation As shown in Figure 4 [Figure 4: see original paper], compared to CK, P fractions changed to varying degrees under 5%, 10%, and 20% addition rates and were clearly separated by the first axis, while the 2% addition rate showed minor changes and was only separated by the second axis. RDA sorting of 12 main environmental factors identified pH (F=66.6, P=0.002), TN (F=6.1, P=0.002), and TP (F=4.1, P=0.012) as the most critical factors influencing P fractions, explaining 74.3%, 5.6%, and 3.3% of P fraction variation, respectively (Figure 4).

Structural equation modeling (SEM) showed that different BC addition rates (Biochar Amounts, BA) affected soil physicochemical properties, which in turn influenced C and N cycling-related enzyme activities (BG, NAG, LAP), ultimately promoting soil P transformation (ACP activity). The model explained 72.4% of P transformation variation (^{2}=7.530, P=0.582, CMIN/df=0.830, NFI=0.964, CFI=1.000, RMSEA<0.050) (Figure 5 [Figure 5: see original paper]A). BA, pH, and soil C:P stoichiometry had direct positive effects on ACP activity, while soil N:P stoichiometry had a direct negative effect. The overall effects on P transformation were ranked as: BA > N:P > C:P > pH (Figure 5B).

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3. Discussion

3.1 Effects of Different Biochar Application Rates on Soil Physicochemical Properties and P Fractions Most BC is produced through pyrolysis of plant biomass, thus preserving many essential nutrient elements required for plant growth and development, and the concentration effect results in high nutrient element content, making it an excellent soil amendment. Numerous studies have found that BC input can change soil physical properties (e.g., porosity, aeration, water content) (Oguntunde et al., 2008; Tian et al., 2013) and chemical properties (e.g., SOC, TN, TP, pH) (Schneider & Haderlein, 2016). In this study, TP content in eucalyptus plantation soil only showed significant effects at the 20% BC addition rate, possibly because BC input promoted the transformation of P fractions in soil TP toward plant-absorbable AP and LP, enhancing soil P availability. MBP is an important active P source in soil that can reflect soil P supply capacity to some extent (Song et al., 2021). The significant increase in MBP content under all BC addition rates in this study indicates that BC input significantly promoted the transformation of soil TP to MBP. This may be because BC's well-developed porosity and large specific surface area facilitate microbial reproduction and growth, and microbial-driven assimilation of organic P and mineralized inorganic P promotes MBP increase (Li et al., 2019). Additionally, microorganisms using organic carbon or other effective nutrients in BC for growth can partially explain the observed increase in microbial biomass (Lehmann et al., 2011). Furthermore, although different BC addition rates had no significant effect on soil TN, NO_3^--N content showed an upward trend with increasing BC input, particularly reaching significant levels at the 20% addition rate—a result also confirmed in Anderson et al. (2011). The reason may be that BC input affects soil nitrification and ammonia oxidation by releasing specific volatile organic substances, adsorbing soil nitrification inhibitors and N substances, and changing soil physicochemical properties.

Based on the different availability and occurrence states of various P fractions in soil for microorganisms and plants according to the Hedley P fractionation method, combined with existing classification methods, this study categorized P fractions into four types: AP, LP, MP, and OP. Among them, AP, LP, and MP are relatively easily absorbed and utilized by plants and can serve as available P sources, while OP is stored within soil minerals or aggregates and is generally inaccessible to plants and microorganisms. Numerous studies have found that soil P transformation is interactively affected by soil physicochemical properties and environmental conditions (Liu & Zhang, 2000; Li et al., 2022). In this study, different BC addition rates had varying effects on P fractions. Redundancy analysis revealed that P fractions were correlated with basic soil physicochemical properties, with pH being one of the most critical factors affecting soil P fraction changes (Figure 4). This is consistent with Tian et al. (2020), possibly because BC addition produces a positive "liming effect," and higher soil pH can increase microbial biomass and activity, ultimately affecting soil P transformation. The improvement of P availability is the main manifestation of BC effects on soil P form transformation. AP and LP contents increased with BC addition rate, with AP reaching significant levels compared to CK at 10% and 20% concentrations, and LP showing significant increases under all BC addition rates. This indicates that different BC addition rates affect soil P solubility (Gundale & Deluca, 2007), promoting the transformation of occluded P to available P (Cai et al., 2014). The main reasons for BC increasing soil available P may include: (1) soluble P salts contained in BC become direct sources of soluble and exchangeable P after soil input (Gundale & Deluca, 2006); (2) when soil pH increases with BC addition, increased negative charges on soil mineral surfaces reduce P adsorption, and BC application may promote P desorption from active metal oxide components, thereby increasing soil P availability (Hosseini et al., 2015); and (3) BC input provides energy substances (e.g., C sources) for soil microorganisms, promoting microbial degradation and immobilization of soil P, ultimately increasing available P content (Huang et al., 2003). Additionally, this study found significant increases in $NaHCO_3$-P, NaOH-P, and HCl-P contents after different BC addition rates, which differs from Xu et al. (2014, 2016) who found no effect of BC addition on soil P fractions and slight decreases in NaOH-P in acidic soils. This discrepancy may be related to soil fertilization status, pH values, different BC addition rates, and pyrolysis temperatures (Chen et al., 2022).

3.2 Effects of Different Biochar Application Rates on Soil Enzyme Activities and P Transformation Many factors affect soil P transformation, and numerous studies have investigated biotic and abiotic factors such as soil weathering degree, different soil types, and fertilization and tillage practices (Guo & Yost, 1998; Shi et al., 2014). Soil microorganisms are major contributors to energy transformation and material cycling in nature, and their metabolic activities can drive soil P turnover and improve soil fertility (Zhang et al., 2014), making them one of the most important factors affecting P transformation. BC can create suitable living spaces for microorganisms involved in soil P transformation, and its input can affect soil P transformation by altering microbial biomass, community structure, and activity (Warnock et al., 2007). Enzymes secreted by microorganisms are key to material cycling in soil ecosystems (Zornoza et al., 2006). In this study, most hydrolytic enzyme activities related to soil C, N, and P cycling showed varying degrees of increase after BC addition (Figure 2), indicating that BC input promoted soil enzyme activity (Paz-Ferreiro et al., 2014). This may be because BC addition increases the content of trace elements such as Zn, Mn, and Cu, which are closely related to soil enzyme structure and activity expression (Li et al., 2014). Second, BC can adsorb and immobilize microorganisms in soil through electrostatic and hydrophobic attraction (Bailey et al., 2011), thereby altering microbial biomass and community structure and benefiting soil enzyme activity improvement (You et al., 2014). Soil acid phosphatase (ACP) activity directly affects soil organic P decomposition, transformation, and bioavailability. In this study, lower BC addition rates (2% and 5%) reduced soil ACP activity, indicating that low BC input inhibited soil P hydrolase activity. The reasons may be that BC-soil binding increased enzyme stability, hindering substrate contact, and that aldehydes and phenols present during BC preparation could have toxic effects on adsorbed enzymes, thus negatively affecting ACP (Yang et al., 2021). The significant positive correlations between ln(BG) and ln(NAG+LAP) with ln(ACP) (Figure 3) indicate that BC input increased soil C and N hydrolase activities (Nasto et al., 2014), promoting C and N transformation. To maintain microbial nutrient element balance, soil microorganisms secrete more ACP to acquire available P to meet their P demands (Xie et al., 2020). The soil C:P ratio can serve as an indicator of microbial potential to mineralize soil organic matter and release P (Liu et al., 2022). In this study, soil C:P ratio had a significant positive effect on ACP (Figure 5), possibly because BC addition increased the soil C:P ratio, causing P limitation and stimulating soil microorganisms to secrete P hydrolase, thereby promoting soil P transformation (You et al., 2020). The N:P ratio can serve as a threshold for determining soil nutrient limitation (Feng et al., 2020). In this study, soil N:P stoichiometry had a significant negative effect on ACP. Since BC itself contains limited N and N can volatilize in gaseous forms (N_2O, NH_3) over incubation time, increasing BC addition rates decreased the soil N:P ratio, causing severe N limitation. Microorganisms then secrete large amounts of N hydrolase to maintain nutrient balance, and improved N availability can affect soil ACP activity by promoting microbial reproduction (Liu et al., 2022). SEM results showed that different BC addition rates affected soil physicochemical properties to varying degrees, thereby increasing enzyme activities related to C and N cycling and ultimately promoting soil P transformation (ACP activity) (Wang et al., 2020). However, the release characteristics of P from BC itself and its long-term slow-release mechanism after soil input remain unclear, requiring more long-term, systematic field experiments to provide scientific support for BC application and promotion in degraded plantation soil restoration in southern China.

4. Conclusion

In this study, all four BC addition rates significantly increased NO_3^--N, MBP, and pH values in eucalyptus plantation soils. Soil AP and LP were only significantly increased at 10% and 20% BC addition rates, while pH, TN, and TP were likely the most important factors altering soil P fractions in eucalyptus plantations. Furthermore, BC primarily improved enzyme activities related to C and N cycling by affecting soil physicochemical properties, thereby enhancing soil P transformation in eucalyptus plantations to some degree, with the highest BC concentration (20%) showing the optimal effect. The pH, C:P, and N:P ratios were the most critical factors driving soil P transformation. In summary, this study demonstrates that BC has potential application value for improving P supply in subtropical eucalyptus plantation soils in China.

References

ACHAT DL, BAKKER MR, AUGUSTO L, et al., 2009. Evaluation of the phosphorus status of P-deficient podzols in temperate pine stands: combining isotopic dilution and extraction methods[J]. *Biogeochemistry*, 92(3): 183-200.

ANDERSON CR, CONDRON LM, CLOUGH TJ, et al., 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus[J]. *Pedobiologia*, 54(5-6): 309-320.

BAILEY VL, FANSLER SJ, SMITH JL, et al., 2011. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization[J]. *Soil Biology and Biochemistry*, 43(2): 296-301.

CAI JZM, ZHAI LM, XI B, et al., 2014. Effect of biochar on Olsen-P and $CaCl_2$-P in different types of soil[J]. *Chinese Journal of Soil Science*, 45(1): 163-168.

CAO J, YAN WD, XIANG WH, et al., 2014. Characteristics of soil phosphorus in different aged stands of Chinese fir plantations in Huitong, Hunan Province[J]. *Acta Ecologica Sinica*, 34(22): 6519-6527.

CHEN WJ, XIA HJ, LIU DF, et al., 2022. Characteristics of biochar and its effects and mechanism on soil properties[J]. *Journal of Environmental Engineering Technology*, 12(1): 161-172.

COSTA MG, GAMA-RODRIGUES AC, GONCALVES JLM, et al., 2016. Labile and non-labile fractions of phosphorus and its transformations in soil under Eucalyptus plantations, Brazil[J]. *Forests*, 7(1): 15.

CROUS KY, OSVALDSSON A, ELLSWORTH DS, 2015. Is phosphorus limiting in a mature Eucalyptus woodland? Phosphorus fertilisation stimulates stem growth[J]. *Plant and Soil*, 391: 25-35.

DEMISIE W, LIU Z, ZHANG M, 2014. Effect of biochar on carbon fractions and enzyme activity of red soil[J]. *Catena*, 121: 214-221.

FENG YH, LIANG WJ, WEI X, et al., 2020. Analysis of soil nutrient characteristics of Larix principis-rupprechtii forests with different altitude gradients in Guandi Mountain[J]. *Journal of West China Forestry Science*, 49(4): 68-73.

FOLTRAN EC, ROCHA JHT, BAZANI JH, et al., 2019. Phosphorus pool responses under different P inorganic fertilizers for a Eucalyptus plantation in a loamy Oxisol[J]. *Forest Ecology and Management*, 435: 170-179.

GAO LY, LIN WP, ZHANG FJ, et al., 2021. Research progress of biochar in improving soil acidification[J]. *Guangdong Agricultural Sciences*, 48(1): 35-44.

GAO YL, FANG F, TANG ZC, et al., 2022. Distribution characteristics of soil phosphorus forms and phosphatase activity at different altitudes in the soil of water-level-fluctuation zone in Pengxi River, Three Gorges Reservoir[J]. *Environmental Science*, 43(10): 4630-4638.

GUNDALE MJ, DELUCA TH, 2006. Temperature and source material influence ecological attributes of ponderosa pine and Douglas-fir charcoal[J]. *Forest Ecology and Management*, 231(1-3): 86-93.

GUNDALE MJ, DELUCA TH, 2007. Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/Douglas-fir ecosystem[J]. *Biology and Fertility of Soils*, 43(3): 303-311.

GUO F, YOST RS, 1998. Partitioning soil phosphorus into three discrete pools of differing availability[J]. *Soil Science*, 163(10): 822-833.

HOSSEINI BS, XU CY, XU Z, et al., 2015. Soil and foliar nutrient and nitrogen isotope composition (δ^{15}N) at 5 years after poultry litter and green waste biochar amendment in a macadamia orchard[J]. *Environmental Science and Pollution Research*, 22(5): 3803-3809.

HUANG M, WU JS, HUANG QY, et al., 2003. Progress in research on microbiological action of soil phosphorus[J]. *Ecology and Environment*, 12(3): 366-370.

HUANG XM, LIU SY, YOU YM, 2014. Effects of N-fixing tree species on soil microbial biomass and community structure of the Second Rotation Eucalyptus Plantations[J]. *Forest Research*, 27(5): 612-620.

JI QK, WANG D, YANG WB, et al., 2021. Effects of long-term phosphorus application on crop yield, phosphorus absorption, and soil phosphorus accumulation in maize-wheat rotation system[J]. *Chinese Journal of Applied Ecology*, 32(7): 2469-2476.

JOHAN, PRISCA D, AHMED, et al., 2021. Phosphorus transformation in soils following co-application of charcoal and wood ash[J]. *Agronomy*, 11(10): 2010.

KLOSS S, ZEHETNER F, WIMMER B, et al., 2014. Biochar application to temperate soils: effects on soil fertility and crop growth under greenhouse conditions[J]. *Journal of Plant Nutrition and Soil Science*, 177(1): 3-15.

LEHMANN J, RILLIG MC, THIES J, et al., 2011. Biochar effects on soil biota—a review[J]. *Soil Biology and Biochemistry*, 43: 1812-1836.

LI F, LIANG X, NIYUNGEKO C, et al., 2019. Effects of biochar amendments on soil phosphorus transformation in agricultural soils[J]. *Advances in Agronomy*, 158: 131-172.

LI X, ZHANG JW, LI LQ, et al., 2014. Effects of Biochar Amendment on Maize Growth and Soil Properties in Huang-Huai-Hai Plain[J]. *Soils*, 46(2): 269-274.

LI XL, HOU XY, MU HB, et al., 2015. P fertilization effects on the accumulation, transformation and availability of soil phosphorus[J]. *Acta Prataculturae Sinica*, 24(8): 218-224.

LI LX, WU GZ, YU ZM, et al., 2022. Experimental study on influencing factors affecting phosphorus availability and total phosphorus leaching in farmland soil in Dagu River Basin[J]. *Journal of Soil and Water Conservation*, 36(2): 337-343.

LI Y, LIU YL, BAI JY, et al., 2019. Responses of soil microbial biomass C and P to different long-term fertilization treatments in the yellow paddy soil[J]. *Chinese Journal of Applied Ecology*, 30(4): 1327-1334.

LIU GS, 1996. *Soil Physical and Chemical Analysis and Profile Description*[M]. Beijing: China Agriculture Press: 24-265.

LIU J, LI J, LONG J, et al., 2022. Altitude dependence of soil ecological stoichiometry and enzyme activities in a Karst region of Southwest China[J]. *Journal of Forest and Environment*, 42(2): 113-122.

LIU JL, ZHANG FH, 2000. The progress of phosphorus transformation in soil and its influencing factors[J]. *Journal of Hebei Agricultural University*, 23(3): 36-45.

LIU S, MENG J, JIANG L, et al., 2017. Rice husk biochar impacts soil phosphorous availability, phosphatase activities and bacterial community characteristics in three different soil types[J]. *Applied Soil Ecology*, 116: 12-22.

LIU YT, WEI J, LI J, 2019. Progress in hydrothermal carbonization of waste biomass and application of biochar in wastewater treatment[J]. *Chemistry and Bioengineering*, 36(1): 1-10.

MURPHY J, RILEY JP, 1962. A modified single solution method for the determination of phosphate in natural waters[J]. *Analytica Chimica Acta*, 27: 31-36.

NASTO MK, ALVAREZ-CLARE S, LEKBERG Y, et al., 2014. Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests[J]. *Ecology Letters*, 17(10): 1282-1289.

NELSON DW, SOMMERS LE, 1982. Total carbon, organic carbon, and organic matter. *Methods of Soil Analysis: Part 2 Chemical and Microbiological*

Properties, 9: 539-579.

OGUNTUNDE PG, ABIODUN BJ, AJAYI AE, et al., 2008. Effects of charcoal production on soil physical properties in Ghana[J]. *Journal of Plant Nutrition and Soil Science*, 171(4): 591-596.

PANDIT NR, MULDER J, HALE SE, et al., 2018. Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil[J]. *Science of the Total Environment*, 625: 1380-1389.

PAZ-FERREIRO J, FU S, MENDEZ A, et al., 2014. Interactive effects of biochar and the earthworm Pontoscolex corethrurus on plant productivity and soil enzyme activities[J]. *Journal of Soils and Sediments*, 14(3): 483-494.

SAIYA-CORK KR, SINSABAUGH RL, ZAK DR, 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil[J]. *Soil Biology and Biochemistry*, 34(9): 1309-1315.

SCHNEIDER F, HADERLEIN SB, 2016. Potential effects of biochar on the availability of phosphorus—mechanistic insights[J]. *Geoderma*, 277: 83-90.

SCHULZ H, DUNST G, GLASER B, 2013. Positive effects of composted biochar on plant growth and soil fertility[J]. *Agronomy for Sustainable Development*, 33(4): 817-827.

SHI Y, WANG ZQ, ZHANG XY, et al., 2014. Effects of nitrogen and phosphorus addition on soil microbial community composition in temperate typical grassland in Inner Mongolia[J]. *Acta Ecologica Sinica*, 34(17): 4943-4949.

SONG KY, MA YP, LI YX, et al., 2021. Effects of Biochar Application on Soil Phosphorus Fractions of Cunninghamia lanceolata Seedlings[J]. *Soils*, 53(6): 1192-1199.

TIAN D, QU ZY, LI B, et al., 2013. Influence of biochar on Sandy Soil hydraulic characteristics parameters and moisture holding properties[J]. *Journal of Irrigation and Drainage*, 32(3): 135-137.

TIAN MY, YU CJ, WANG JK, et al., 2020. Effect of nitrogen additions on soil pH, phosphorus contents and phosphatase activities in grassland[J]. *Chinese Journal of Applied Ecology*, 31(9): 2985-2992.

VALADARES SV, NEVES JCL, LEITE HG, et al., 2020. Predicting phosphorus use efficiency and allocation in eucalypt plantations[J]. *Forest Ecology and Management*, 460: 117859.

VANCE ED, BROOKES PC, JENKINSON DS, 1987. An extraction method for measuring soil microbial biomass C[J]. *Soil Biology and Biochemistry*, 19(6): 703-707.

WANG M, ZHOU HY, YU FY, et al., 2021. Dynamic changes of undergrowth species diversity and biomass of Eucalyptus robusta plantations at different ages[J]. *Bulletin of Botanical Research*, 41(4): 496-505.

WANG T, WAN XH, WANG L, et al., 2020. Effects of broadleaved tree plantation on soil phosphorus fractions and availability in different soil layers in a logged Cunninghamia lanceolata woodland[J]. *Chinese Journal of Applied Ecology*, 31(4): 1088-1096.

WARNOCK DD, LEHMANN J, KUYFER TW, et al., 2007. Mycorrhizal responses to biochar in soil–concepts and mechanisms[J]. *Plant and Soil*, 300(1): 9-20.

WEN YG, ZHOU XG, ZHU HG, et al., 2019. Theoretical exploration and practices of ecological management in Eucalyptus Plantations[J]. *Guangxi Sciences*, 26(2): 159-175.

WU Y, XU G, LV YC, et al., 2014. Effects of biochar amendment on soil physical and chemical properties: current status and knowledge gaps[J]. *Advances in Earth Science*, 29(1): 68-79.

XIE H, ZHANG QF, ZENG QX, et al., 2020. Nitrogen application drives the transformation of soil phosphorus fractions in Cunninghamia lanceolata plantation by changing microbial biomass phosphorus[J]. *Chinese Journal of Ecology*, 39(12): 3934-3942.

XU G, SUN JN, SHAO HB, et al., 2014. Biochar had effects on phosphorus sorption and desorption in three soils with differing acidity[J]. *Ecological Engineering*, 62: 54-60.

XU G, ZHANG Y, SUN J, et al., 2016. Negative interactive effects between biochar and phosphorus fertilization on phosphorus availability and plant yield in saline sodic soil[J]. *Science of the Total Environment*, 568: 910-915.

YANG K, WANG YY, DING AZ, 2021. Stability of biochar-remediated contaminated soil from a lead mine site[J]. *Journal of Agro-Environment Science*, 40(12): 2715-2722.

YANG X, POST WM, 2011. Phosphorus transformations as a function of pedogenesis: A synthesis of soil phosphorus data using Hedley fractionation method[J]. *Biogeosciences*, 8(10): 2907-2916.

YOU Y, WANG J, HUANG X, et al., 2014. Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover[J]. *Ecology and Evolution*, 4(5): 633-647.

YOU Y, XU H, WU X, et al., 2020. Native broadleaf tree species stimulate topsoil nutrient transformation by changing microbial community composition and physiological function, but not biomass in subtropical plantations with low P status[J]. *Forest Ecology and Management*, 477: 118496.

ZHANG L, WU N, WU Y, et al., 2009. Soil phosphorus form and fractionation scheme: A review[J]. *Chinese Journal of Applied Ecology*, 20(7): 1775-1782.

ZHANG SH, HUANG J, LUO RZ, et al., 2014. Effect of adding different amounts of wheat straw and phosphorus on soil microorganism community[J].

Chinese Journal of Applied Ecology, 25(3): 797-802.

ZHANG W, ZHANG Y, AN Y, et al., 2021. Phosphorus fractionation related to environmental risks resulting from intensive vegetable cropping and fertilization in a subtropical region[J]. *Environmental Pollution*, 269: 116098.

ZHAO ZZ, WANG XL, LI HB, et al., 2021. Slow-release property and soil remediation mechanism of biochar-based fertilizers[J]. *Journal of Plant Nutrition and Fertilizers*, 27(5): 886-897.

ZORNOZA R, GUERRERO C, SOLERA MJ, et al., 2006. Assessing air-drying and rewetting pre-treatment effect on some soil enzyme activities under Mediterranean conditions[J]. *Soil Biology and Biochemistry*, 38(8): 2125-2134.

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