

## Effects of Biochar Application on Soil Enzyme Activity in Eucalyptus Plantations in Northern Guangxi (Postprint)

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### Abstract

This study investigated the response characteristics of soil enzyme activity to biochar input to provide a theoretical reference for the rational application of eucalyptus branch biochar. Based on a field experiment in eucalyptus plantations in northern Guangxi, biochar prepared from eucalyptus plantation harvesting residue branches under anaerobic conditions at 500°C was applied at mass fractions of 0 (CK), 0.5% (T1), 1% (T2), 2% (T3), 4% (T4), and 6% (T5), and changes in soil enzyme activity under different treatments were analyzed after one year of input. The results showed that: (1) soil enzyme content decreased with vertical soil depth; (2) the contents of urease, catalase, -glucosidase, and dehydrogenase in each soil layer increased with increasing biochar application rate, reaching the highest values at a biochar application rate of 6%; (3) the contents of acid phosphatase, sucrase, leucine aminopeptidase, and cellobiosidase exhibited a trend of first increasing and then decreasing with increasing biochar application rate, with acid phosphatase and leucine aminopeptidase reaching their highest values at a biochar application rate of 2%, while sucrase and cellobiosidase reached their highest values at 4%. Overall, the application of eucalyptus branch biochar enhanced soil enzyme activity in eucalyptus plantations to varying degrees. These research results provide a scientific basis for the resource utilization pathways of forestry waste-derived biochar and its application in eucalyptus plantations.

## Full Text

### Effects of Biochar Application on Soil Enzyme Activities in Eucalyptus Plantations in North Guangxi

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**Abstract:** This study investigated the response characteristics of soil enzyme activities to biochar input to provide theoretical references for the rational application of Eucalyptus branch biochar. Based on field experiments in a Eucalyptus plantation in North Guangxi, biochar was prepared anaerobically at 500 °C from Eucalyptus harvesting residues (branches). Biochar was applied at mass fractions of 0 (CK), 0.5% (T1), 1% (T2), 2% (T3), 4% (T4), and 6% (T5), and soil enzyme activities were analyzed one year after application. The results showed: (1) Soil enzyme content decreased with soil depth. (2) The contents of urease, catalase,  $\alpha$ -glucosidase, and dehydrogenase in each soil layer increased with biochar application rate, reaching maximum values at 6% application. (3) Acid phosphatase, sucrase, leucine aminopeptidase, and cellobioglucosidase contents initially increased then decreased with increasing biochar application, with acid phosphatase and leucine aminopeptidase peaking at 2% biochar, while sucrase and cellobioglucosidase peaked at 4%. Overall, Eucalyptus branch biochar application enhanced soil enzyme activities in the Eucalyptus plantation to varying degrees. These findings provide a scientific basis for the resource utilization of forestry waste-derived biochar and its application in Eucalyptus plantations.

**Keywords:** biochar, soil enzyme activity, Eucalyptus plantation, North Guangxi, field experiment

Biochar is the solid residue produced from pyrolysis of biomass under anaerobic conditions, with its properties influenced by feedstock sources and pyrolysis conditions (Campos et al., 2020). Rich in carbon content, biochar has low bulk density, large specific surface area, loose and porous structure, and strong ad-

sorption capacity. Biochar addition can increase soil organic carbon content, regulate and maintain soil moisture and aeration, improve soil fertility, and consequently promote plant growth (Wang et al., 2020). Research has demonstrated that biochar can be widely used as a novel soil amendment for soil remediation (He et al., 2015; Duan et al., 2020; Xu et al., 2020; Wang et al., 2021).

Soil enzymes originate from secretions of soil animals, plants, and microbial cells, as well as decomposition of residues, representing one of the most active soil components. They play positive roles in organic matter decomposition (Guan, 1986) and can indicate changes in soil quality (Xu et al., 2017). Based on their functions and catalytic reaction types, soil enzymes are primarily classified as hydrolases, lyases, oxidoreductases, and transferases (Guan, 1986). Oxidoreductases catalyze oxidation-reduction reactions in soil and are important for energy transfer and substance metabolism, mainly including catalase and dehydrogenase. Hydrolases decompose proteins and other substances into forms easily absorbed by plants, including sucrase, urease, phosphatase, peptidase, and cellulase. Studying changes in soil enzyme activities can better reflect the effects of biochar input on soil microecology. Previous studies have shown that biochar application in continuous cotton cropping systems on gray desert soil and aeolian sandy soil in Xinjiang improved rhizosphere soil nutrients and microbial diversity (Gu et al., 2014). However, other research reported that branch-derived biochar enhanced enzyme activities related to nitrogen and phosphorus cycling in loam and sandy soils while reducing carbon cycle-related enzyme activities in loam soil (Bailey et al., 2011; Shang et al., 2016). Current research on biochar effects on soil enzyme activities has focused primarily on laboratory incubation or short-term field experiments, with inconsistent results (Castaldi et al., 2011). Thus, biochar effects on soil enzyme activities vary considerably depending on feedstock sources and soil types. Therefore, investigating changes in soil enzyme activities after biochar application through field experiments is necessary to deeply reveal the amelioration effects of biochar on soils.

Eucalyptus, as a fast-growing tree species, has a long planting history in Guangxi, with continuously expanding plantation areas that promote local economic development. However, ecological problems such as soil fertility decline exist in Eucalyptus plantation management (Huang et al., 2014; Wen et al., 2019), and soil quality and fertility levels need further improvement. Eucalyptus plantations generate substantial forestry waste during management. Preparing biochar from this waste and returning it to the field could yield significant ecological and economic benefits if Eucalyptus branch biochar plays a positive role in Eucalyptus plantations. While biochar research in agriculture is extensive, studies on biochar application in Eucalyptus plantations are limited, and the effects of biochar application on soil enzyme activities in Eucalyptus plantations remain unclear. Therefore, this study used Eucalyptus plantation harvesting residues (branches) as raw material to prepare biochar through high-temperature anaerobic pyrolysis, applied it to Eucalyptus plantation soil at different mass fractions, and examined its effects on soil catalase, urease, and other enzyme activities to identify the optimal biochar application rate

for promoting soil enzyme activities. The results are expected to provide theoretical references for resource utilization of forestry waste-derived biochar and sustainable management of Eucalyptus plantations.

## 1. Study Area Overview

The experimental site was located at the Guangxi State-owned Huangmian Forestry Farm (109°43'46" E, 24°37'25" N), characterized by low mountains and hills. The soil type was primarily mountainous yellow-red soil and red soil. Detailed descriptions are provided in Duan et al. (2020).

## 2. Materials and Methods

### 2.1 Field Plots and Soil Sampling

Biochar was produced from Eucalyptus plantation harvesting residues (branches) collected from the Huangmian Forestry Farm experimental site and surrounding areas. The material was pyrolyzed under high temperature (500 °C) and anaerobic conditions by Jining Dehanqi Mechanical Engineering Technology Co., Ltd. Specific biochar properties are described in detail in Duan et al. (2020).

A randomized block experiment was initiated in March 2017 in the Eucalyptus plantation. Following the mass percentage and complete mixing method referenced in Guo et al. (2015), biochar was applied at rates of CK (0%), T1 (0.5%), T2 (1%), T3 (2%), T4 (4%), and T5 (6%). The experiment included three replicates per treatment, totaling 18 experimental plots, each measuring 8 m × 8 m. Soil samples were collected in March 2018 at 10 cm intervals to a depth of 30 cm using a five-point sampling method (Duan et al., 2020). Soil samples were air-dried for determination of soil enzyme activities and physicochemical properties.

### 2.2 Soil Enzyme Activity Analysis

Soil enzyme activities were determined following the methods of Guan (1986), with three replicates per sample. Urease (URE,  $\text{mg} \cdot \text{g}^{-1}$ ), sucrase (SUC,  $\text{mg} \cdot \text{g}^{-1}$ ), acid phosphatase (ACP,  $\text{mg} \cdot \text{g}^{-1}$ ), catalase (CAT,  $\text{mL} \cdot \text{g}^{-1}$ ), dehydrogenase (DHA,  $\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ ), and  $\alpha$ -glucosidase (BG,  $\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ ) activities were measured using phenol sodium colorimetry, 3,5-dinitrosalicylic acid colorimetry, disodium phenyl phosphate colorimetry, potassium permanganate titration, triphenyl tetrazolium chloride reduction, and nitrophenol colorimetry methods, respectively. Soil cellobioglucosidase (CB,  $\text{nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ ) and leucine aminopeptidase (LAP,  $\text{nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ ) were determined using microplate fluorometry (Bell et al., 2013).

## 2.3 Soil Physicochemical Property Analysis

Soil bulk density (SBD) was measured using the ring knife method, and soil water content (SWC) by the drying method. Soil pH was determined using a pH meter. Soil porosity (SP) and total soil porosity (TSP) were calculated from density and bulk density. Soil organic carbon (SOC) was measured with a Shimadzu 5000A TOC analyzer. Total phosphorus (TP) was determined by molybdenum-antimony anti-colorimetry (BUV-1600 UV-Vis spectrophotometer). Total potassium (TK) was measured by flame photometry after sulfuric acid-perchloric acid digestion. Available nitrogen (AN) was determined by alkaline hydrolysis diffusion method. Available phosphorus (AP) was extracted with sodium bicarbonate and measured by molybdenum-antimony anti-colorimetry. Available potassium (AK) was measured by flame photometry (Cole Parmer flame photometer, USA). Cation exchange capacity (CEC) was determined using  $1 \text{ mol} \cdot \text{L}^{-1}$  ammonium acetate exchange method. Electrical conductivity (EC) was measured with a conductivity meter (DDS-307A) at a soil-to-water ratio of 5:1. Exchangeable acid (E-ac), exchangeable aluminum (E-al), and exchangeable hydrogen (E-hy) were extracted with  $1 \text{ mol} \cdot \text{L}^{-1}$  KCl and titrated with  $0.02 \text{ mol} \cdot \text{L}^{-1}$  NaOH (Lu, 2000). Following the extraction method of Ma et al. (2007), exchangeable sodium (E-na), exchangeable calcium (E-ca), and exchangeable magnesium (E-ma) were determined by inductively coupled plasma optical emission spectrometry (ICP-7400, ThermoFisher Scientific).

## 2.4 Data Processing

Charts were prepared and data were processed using Excel 2010 and SPSS 23.0 software. One-way ANOVA, LSD multiple comparisons ( $\alpha = 0.05$ ), and Pearson correlation analysis were performed on soil enzyme activities under different treatments.

## 3. Results

### 3.1 Changes in Soil Catalase and Urease Under Different Treatments

As shown in Figure 1, compared with the 0–30 cm soil layer in the CK treatment, catalase and urease contents consistently increased with biochar application rate, with increases of 7.97%–56.46% and 5.48%–31.45%, respectively, reaching maximum values in the T5 treatment. Under the same treatment, both enzymes showed significant decreasing trends with soil depth. Among different treatments within the same soil layer, catalase in the 0–10 cm layer showed significant differences ( $P < 0.05$ ) between all treatments except between T1 and CK and between T1 and T2. In the 10–20 cm layer, no significant differences were observed between T4 and T5 for either catalase or urease, while all other treatment pairs differed significantly. In the 20–30 cm layer, both catalase and urease showed significant differences among all treatments ( $P < 0.05$ ).

**Note:** Different lowercase letters indicate significant differences between differ-

ent treatments in the same soil layer ( $P < 0.05$ ). Different capital letters indicate significant differences between different soil layers in the same treatment ( $P < 0.05$ ). The same notation applies below.

### 3.2 Changes in Soil Dehydrogenase and $\alpha$ -Glucosidase Under Different Treatments

Figure 2 shows that compared with the 0–30 cm soil layer in the CK treatment, biochar application increased dehydrogenase and  $\alpha$ -glucosidase contents by 53.51%–202.33% and 12.12%–83.09%, respectively, with maximum values in the T5 treatment. Both enzymes showed significant decreasing trends with soil depth across all treatments. Among different treatments within the same soil layer, dehydrogenase in the 0–10 cm layer showed significant differences between all treatments except between T1 and T2 and between T3 and T4. For  $\alpha$ -glucosidase in the 0–10 cm layer, significant differences were observed between all treatments except between T2 and CK and between T2 and T1. In the 10–20 cm layer, dehydrogenase showed no significant differences between CK and T1 or between T3 and T4, while  $\alpha$ -glucosidase showed no significant differences between T1 and T2 or between T1 and T3 ( $P > 0.05$ ), but differed significantly from other treatments. In the 20–30 cm layer, dehydrogenase showed no significant differences between T4 and T2 or between T4 and T3 ( $P > 0.05$ ), while  $\alpha$ -glucosidase showed significant differences among all treatments except between T1 and T2 ( $P < 0.05$ ). Overall, biochar application had particularly pronounced effects on soil dehydrogenase and  $\alpha$ -glucosidase.

### 3.3 Changes in Soil Acid Phosphatase and Sucrase Under Different Treatments

Figure 3 shows that compared with the 0–30 cm soil layer in the CK treatment, acid phosphatase content followed the order  $T5 < CK < T4 < T1 < T2 < T3$ , peaking in the T3 treatment, while sucrase content followed the order  $CK < T1 < T2 < T3 < T5 < T4$ , peaking in the T4 treatment. Both enzymes showed significant decreasing trends with soil depth. Among different treatments within the same soil layer, acid phosphatase in the 0–10 cm layer showed no significant differences between T4 and CK or between T4 and T1 ( $P > 0.05$ ), while sucrase showed no significant difference between T4 and T5 ( $P > 0.05$ ), with all other treatments differing significantly ( $P < 0.05$ ). In the 10–20 cm layer, sucrase showed no significant differences between CK and T1 or between T4 and T5 ( $P > 0.05$ ), while all other treatments differed significantly. In the 20–30 cm layer, acid phosphatase showed no significant difference between CK and T4 ( $P > 0.05$ ), with all other treatments differing significantly ( $P < 0.05$ ), while sucrase showed significant differences among all treatments ( $P < 0.05$ ).

### 3.4 Changes in Soil Leucine Aminopeptidase and Cellobioglucosidase Under Different Treatments

Figure 4 shows that compared with the 0–30 cm soil layer in the CK treatment, leucine aminopeptidase content followed the order  $T1 < CK < T5 < T4 < T2 < T3$ , peaking in the T3 treatment, while cellobioglucosidase content followed the order  $CK < T1 < T2 < T3 < T5 < T4$ , peaking in the T4 treatment. Both enzymes showed significant decreasing trends with soil depth. Among different treatments within the same soil layer, leucine aminopeptidase in the 0–10 cm layer showed no significant differences between CK and T1, between CK and T5, or between T4 and T5 ( $P > 0.05$ ), while cellobioglucosidase showed no significant difference between T4 and T5 ( $P > 0.05$ ), with all other treatments differing significantly. In the 10–20 cm layer, leucine aminopeptidase showed no significant differences between CK and T1 or between T2 and T4 ( $P > 0.05$ ), while cellobioglucosidase showed no significant difference between T4 and T5 ( $P > 0.05$ ), but differed significantly from other treatments ( $P < 0.05$ ). In the 20–30 cm layer, both leucine aminopeptidase and cellobioglucosidase showed no significant differences between CK and T1 ( $P > 0.05$ ), but differed significantly from other treatments ( $P < 0.05$ ).

### 3.5 Correlation Characteristics Between Soil Enzyme Activities and Physicochemical Properties

Table 1 shows that CAT, URE, DHA, BG, ACP, SUC, LAP, and CB were all extremely significantly positively correlated with CEC, E-ca, E-ma, SWC, SP, TSP, SOC, TP, TK, AP, AK, and AN ( $P < 0.01$ ). pH and EC were significantly positively correlated with ACP ( $P < 0.05$ ) and extremely significantly positively correlated with other soil enzymes ( $P < 0.01$ ). SBD was extremely significantly positively correlated with ACP ( $P < 0.01$ ) and significantly correlated with LAP ( $P < 0.05$ ). E-ac and E-al showed no significant correlations with CAT, SUC, DHA, or BG ( $P > 0.05$ ). E-hy was significantly positively correlated with SUC but showed no significant correlations with CAT or DHA ( $P > 0.05$ ). E-na was extremely significantly positively correlated with SUC, URE, ACP, BG, CB, and LAP ( $P < 0.01$ ). These results indicate close relationships between soil enzyme activities and soil physicochemical properties, with soil enzyme activities influenced by multiple factors, and soil organic carbon having particularly notable effects on enzyme activities.

**Note:** +\* indicates significant positive correlation ( $P < 0.05$ ); +\*\* indicates extremely significant positive correlation ( $P < 0.01$ ); +n indicates no significant positive correlation ( $P > 0.05$ ); -n indicates no significant negative correlation ( $P > 0.05$ ).

## 4. Discussion

Soil sucrase reflects the accumulation and transformation status of soil organic matter, while catalase primarily participates in the degradation of lignin and



phenolic substances, promoting the formation of soil humus (Burns et al., 2013).  $\alpha$ -Glucosidase is a major polysaccharide-decomposing enzyme. Catalase and cellulase play important roles in the decomposition and transformation of soil organic carbon (Schimel & Weintraub, 2003). Soil dehydrogenase can reflect active microbial biomass and characteristics of organic matter degradation in soil systems, serving as an indicator of soil microbial degradation performance. These enzymes are primarily involved in carbon cycling in soil.

Bamminger et al. (2013) demonstrated that corn straw biochar application significantly enhanced  $\alpha$ -glucosidase activity in forest soils. Previous studies have reported that biochar application decreased catalase and cellulase activities (Lehmann et al., 2011), while application of activated sludge biochar to sandy loam and red soil significantly enhanced dehydrogenase and  $\alpha$ -glucosidase activities (Demisie et al., 2014). Jin et al. (2018) found that intercropping combined with biochar addition improved soil catalase, dehydrogenase, and urease activities more effectively. Gao et al. (2019) discovered that biochar application in Chinese cabbage-cultivated soil increased cellulase and sucrase activities. Research has shown that application of corn and rapeseed biochar to tobacco-growing soil promoted urease, sucrase, protease, and  $\alpha$ -glucosidase activities, with effects varying across growth periods (Du et al., 2021). Similar to these findings, our study showed that dehydrogenase and  $\alpha$ -glucosidase contents increased gradually with biochar application rate within the same soil layer, while decreasing significantly with soil depth under the same treatment, indicating that Eucalyptus branch biochar promoted activities of enzymes related to soil carbon transformation.

Hu et al. (2019) reported that addition of Chinese fir biochar to southern red soil in Chinese fir plantations had no significant effect on catalase. He et al. (2020) applied biochar to grape seedling soil and found that catalase and sucrase activities increased with biochar application rate. Hydrogen peroxide generally exerts toxic effects on beneficial soil microorganisms, but catalase promotes hydrogen peroxide decomposition. In our experiment, catalase activity across treatments followed the order  $T5 > T4 > T3 > T2 > T1$ , indicating that biochar application enhanced soil catalase activity and promoted hydrogen peroxide decomposition. Additionally, the loose and porous structure of Eucalyptus branch biochar may adsorb hydrogen peroxide, collectively reducing potential harm to soil. Thus, biochar application improved soil quality in Eucalyptus forests.

Urease is a key enzyme in urea transformation and can indicate soil nitrogen supply capacity. Soil leucine aminopeptidase is a class of proteases that hydrolyze peptide chains with N-terminal leucine (Guan, 1986). Previous studies have shown that wheat straw biochar significantly increased urease activity in podzol soil (Oleszczuk et al., 2014), and oak-bamboo mixed biochar application to red soil enhanced urease activity (Demisie et al., 2014). Wang et al. (2019) found that different corn straw biochar application rates promoted soil urease, sucrase, alkaline phosphatase, and catalase activities, with urease showing more pronounced enhancement at high application rates. Wang et al. (2021) com-



pared single biochar application with combined biochar and organic fertilizer application in cultivated red soil, finding that while single biochar application significantly increased soil urease, sucrase, and catalase activities, the combined application further promoted enzyme activities. In our study, catalase content increased gradually with biochar application rate within the same soil layer, indicating that biochar application significantly enhanced soil urease activity. Moreover, soil urease was closely related to available nitrogen ( $P < 0.01$ ), suggesting that exogenous Eucalyptus branch biochar improved soil nitrogen supply capacity.

Acid phosphatase is an important indicator for evaluating phosphorus transformation. Demisie et al. (2015) reported that soil acid phosphatase activity decreased with increasing biochar application. Li et al. (2019) added different biochar rates to coastal saline-alkali soil and found that urease activity increased with biochar rate, while acid phosphatase showed an initial increase followed by a decrease. Similar to these results, our study found that acid phosphatase activity initially increased then decreased with increasing Eucalyptus branch biochar application rate. Lehmann et al. (2011) demonstrated that biochar is rich in P, K, Mg, and other elements, promoting soil microbial growth and consequently increasing soil enzyme activities. These findings indicate that due to differences in biochar application rates and inherent elemental contents, biochar from different sources has varying effects on soil enzyme activities. In our study, returning Eucalyptus branch biochar to the field improved soil physicochemical properties, promoted soil enzyme activities, and provided nutrients that could serve as substrates for enzyme-producing microorganisms. Combined with its porous structure and adsorption capacity, biochar influenced the quantity of reaction substrates in soil, promoted microbial activity, enhanced enzymatic reactions, and consequently increased soil enzyme activities. Due to biochar's unique properties, soil enzyme activities changed to varying degrees after biochar addition.

Sun et al. (2016) found that catalase activity was affected by soil pH and organic matter content, being positively correlated with soil pH. Zheng et al. (2019) applied wheat straw biochar to red soil and found that soil urease and  $\alpha$ -glucosidase were significantly positively associated with pH ( $P < 0.05$ ), while negatively correlated with acid phosphatase ( $P < 0.05$ ), indicating that biochar regulated soil biochemical processes by affecting pH and that enzyme activities were linked to soil nutrients. Xu et al. (2019) found that biochar application in paddy soil increased urease and acid phosphatase activities, with acid phosphatase activity being extremely significantly positively correlated with soil bulk density. Wang et al. (2019) reported that biochar application increased soil organic carbon, total nitrogen, available phosphorus, and available potassium contents. Biochar itself is rich in carbon, and its addition to soil may increase carbon sources, while its promotion of nitrogen may result from enhanced enzyme activities. Our study found that biochar application increased soil phosphatase and sucrase activities, which was closely associated with increased soil organic matter and available phosphorus. Duan et al. (2020) reported that high biochar applica-

tion rates resulted in higher soil available phosphorus contents, indicating that acid phosphatase promoted soil phosphorus accumulation. In our study, soil  $\alpha$ -glucosidase was extremely significantly positively correlated with soil organic carbon content, suggesting that  $\alpha$ -glucosidase changes were related to organic carbon levels, consistent with Duan et al. (2020) findings that biochar addition increased soil organic carbon content. Our results demonstrate that Eucalyptus branch biochar application affected soil enzyme activities, with changes varying by application rate, and that enzyme activities indicated promotion of soil nutrient contents.

Research has shown that 0.5% biochar (corn straw, 450 °C) promoted soil cellulose hydrolase and  $\alpha$ -glucosidase activities, while 1% biochar addition inhibited these enzyme activities (Wang et al., 2015). In purple soil, 4% biochar application resulted in the highest sucrase content (Li, 2016). Wang et al. (2020) added biochar to cadmium-contaminated soil and found that urease and sucrase increased significantly at 5% biochar application, while acid phosphatase and catalase decreased with application rate and stabilized at 5% biochar. Banana stem-leaf biochar increased enzyme activities in acidified banana orchard soil, with 3% application showing better soil fertility effects (Xu et al., 2020). Similar to these studies, our results showed that dehydrogenase, catalase,  $\alpha$ -glucosidase, and urease contents increased with biochar application rate, reaching maximum values at 4–6% biochar, with high application rates showing significant enhancement effects on these enzymes in Eucalyptus plantation soil. In contrast, sucrase, cellobioglucosidase, leucine aminopeptidase, and acid phosphatase contents initially increased then decreased with biochar application rate. Thus, soil enzyme activities indicate changes in soil physicochemical properties, and Eucalyptus plantation soil enzyme activities are collectively influenced by biochar properties, soil and crop types, environmental factors (soil nutrients, acidity/alkalinity, etc.), and anthropogenic factors (fertilization, irrigation, management practices, etc.). Elzobair et al. (2016) suggested that soil microbial utilization of nutrients in biochar affects enzyme activities. Wang et al. (2021) found that biochar significantly increased soil pH and carbon and nitrogen contents, with nitrogen showing significant correlations with several hydrolases, as nitrogen affects enzyme activities by influencing soil microbial quantity and function. In our study, Eucalyptus branch biochar changed soil pH and other physicochemical properties through its high nutrient content, indirectly affecting mineral element availability, improving the soil environment in Eucalyptus plantations, and consequently influencing enzyme activities. Additionally, biochar may affect enzyme activities by promoting soil microbial reproduction, growth, and metabolism.

Despite minor differences among enzyme types, biochar application at rates above 2% increased soil enzyme activities compared with the control. These enzymes are closely related to carbon, nitrogen, and phosphorus transformation, further demonstrating that biochar application in Eucalyptus plantations provides good soil fertility retention capacity.

In summary, Eucalyptus branch biochar application in Eucalyptus plantations significantly affected soil enzyme activities. Urease, catalase, dehydrogenase, and  $\alpha$ -glucosidase contents increased with biochar application rate across soil layers, reaching maximum values at 6% application. Leucine aminopeptidase and acid phosphatase peaked at 2% application, showing an initial increase followed by decrease. Cellobioglucosidase and sucrase contents peaked at 4% application. The effects of biochar on enzyme activities gradually weakened with increasing soil depth. Soil enzyme activities were closely related to soil physicochemical properties. Overall, biochar application in Eucalyptus plantations in North Guangxi improved soil physicochemical properties and enhanced soil enzyme activities.

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