

Root architectural plasticity and rhizosphere phosphatase hotspots jointly enhance phosphorus acquisition in *Camellia oleifera* on amendment-treated acidic red soils

Huimin Yin

Yuhua Li

Xinyi Zhang

Shanshan Su

Yuhong Li

Lichao Wu

wulichao@sina.com

Central South University of Forestry and Technology <https://orcid.org/0000-0002-5943-7210>

Xiaojun Jiang

Jun Yuan

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3 **Authors:** Huimin Yin^a, Yuhua Li^b, Xinyi Zhang^a, Shanshan Su^a, Yuhong Li^a, Lichao Wu^{a*},
4 Xiaojun Jiang^{c*}, Jun Yuan^{a*}

5 ^a Key Laboratory of Soil and Water Conservation and Desertification Combating of Hunan
6 Province, School of Forestry, School of Soil and Water Conservation, Central South
7 University of Forestry and Technology, Changsha, 410004, China;

8 ^b Liangfengjiang National Forest Park, Nanning, Guangxi, 530000, China;

9 ^c YongZhou Vocational Technical College, Hunan, 425100, China.

10 *** Corresponding author:**

11 Lichao Wu, Tel: +86 731-856 234 52; E-mail: wulichao@sina.com;

12 Jun Yuan, Tel: +86 731-856 234 50; E-mail: yuanjun@csuft.edu.cn;

13 Xiaojun Jiang, Tel: +86 746 636 7068; E-mail: 13973490169@163.com.

14

Abstract

Aims Severe soil acidification and phosphorus (P) deficiency limit plant productivity in subtropical red soil regions. These conditions restrict root development and reduce P availability for *Camellia oleifera*. We aimed to elucidate the poorly understood rhizosphere mechanisms governing P acquisition.

Methods *In situ* soil zymography was combined with root morphological analysis to investigate P acquisition strategies and spatial phosphatase patterns under the joint application of organic amendments and P fertilizers.

Results Amendment application increased soil pH from 4.09 to 7.09 and cation exchange capacity. This reduced root lignin content from 27.55% to 11.70%, increasing root branching (144%) and total root length (217%). Combined treatments expanded spatial phosphatase activity: plant-derived acid phosphatase concentrated at the rhizoplane (424.78% increase), while microbial alkaline phosphatase extended into non-rhizosphere regions. These patterns accompanied soil P fraction shifts, decreasing HCl-P while increasing citrate-P and enzyme-P pools. Path modeling revealed soil nutrients directly affected root morphology (path coefficient 0.62) and bioavailable P pools (0.82). Bioavailable P was positively associated with enzymatic hotspots (0.85) and plant P uptake (0.30). Combined T2 amendment with moderate-to-high P supply maximized P bioavailability and plant uptake.

Conclusions These findings provide a process-based framework emphasizing how root architectural plasticity and rhizosphere enzymatic hotspots jointly enhance plant P acquisition.

Keywords: *Camellia oleifera*, Amendment, Phosphorus mobilization, Root morphology, Soil zymography

Introduction

Widespread phosphorus (P) deficiency in global red soils represents a major limitation on agricultural productivity and ecosystem functioning (Chen et al., 2023; Liu et al., 2023; Zhou et al., 2025). Under prolonged weathering in hot and humid climates, these soils undergo extensive desilication and the accumulation of iron (Fe) and aluminum (Al) oxides, accompanied by pronounced acidification. High concentrations of reactive Fe and Al oxides in red soils readily form stable precipitates with orthophosphate, resulting in P fixation and extremely low biological availability despite abundant total P reserves (He et al., 2021; Scherwietes et al., 2025). In southern China, long-term inappropriate fertilization practices have further increased the severity of these restrictions across red soil ecosystems (Xi et al., 2025). Current amelioration strategies mainly rely on lime (Li et al., 2022) or biochar (Fan et al., 2020) application to alleviate soil acidification (Siedt et al., 2021). However, such approaches often overlook the stoichiometric balance of nutrients. Organic amendments derived from plant residues typically contain high carbon and nitrogen but limited phosphorus, which can aggravate P deficiency through microbial immobilization (Fan et al., 2020). Overcoming the limitations of conventional amendments to simultaneously restore nutrient balance and alleviate P deficiency in acidified soils remains a critical research priority.

Camellia oleifera is recognized as one of the four major woody oil species worldwide, producing high-quality edible seed oil and holding significant economic and ecological

value, often referred to as the Eastern olive oil (Gao et al., 2024). Its cultivation is largely confined to strongly acidic red soils, where long-term lime application and conventional management practices have not effectively restored soil quality or alleviated P deficiency (He et al., 2011). As a typical acidophilic species, *C. oleifera* has evolved unique adaptive strategies to navigate acidic environments, heavily relying on the intensive exudation of organic acids and acid phosphatase (ACP) to mobilize recalcitrant P fractions (Zheng et al., 2021). However, in severely acidified Ultisols (pH < 4.5), this active P-foraging strategy is severely hindered by extreme proton stress (Kopittke et al., 2015). To survive this chronic acidification, *C. oleifera* resorts to a more costly defensive strategy centered on root lignification (Yang et al., 2011; Zhou et al., 2019). While this physiological adjustment reinforces cell-wall structural integrity against acid stress, it simultaneously immobilizes P in the apoplast through interactions with pectin and hemicellulose. This process drastically reduces the metabolically active P pool available for root expansion and extracellular enzymatic activity (Yang et al., 2011; Zhou et al., 2019). Consequently, pronounced alternate bearing cycles and persistently low productivity driven by P limitation remain unresolved challenges in *C. oleifera* plantations (Zhang et al., 2021).

Applying alkaline amendments to these soils presents an ecological and physiological challenge. While such amendments ameliorate extreme acid stress and reduce the need for P-immobilizing root lignification, they simultaneously alter the native highly acidic environment that *C. oleifera* typically uses for P mining (Yang et al., 2011). Because plant-derived ACP is the primary agent of organic P hydrolysis under strongly acidic conditions owing to its low pH optimum, whereas microbial-derived alkaline phosphatase (ALP)

becomes active only once soil pH rises following amendment application, these two enzymatic systems are governed by fundamentally different pH sensitivities and cellular origins (Zheng et al., 2021; Spohn and Kuzyakov, 2013). Amendment-driven pH elevation is therefore expected to trigger a spatial decoupling of these two enzymatic networks: *C. oleifera* retains a localized acidic micro-niche at the rhizoplane to sustain ACP-mediated P mobilization, while microbial ALP activity expands outward into the neutralized bulk soil (Hinsinger et al., 2003; Wen et al., 2019). Plants further cope with the combined stresses of acidification and P deficiency by modifying root morphological traits, such as increased lateral root proliferation and root hair development, which expand the absorptive surface area under P-limited conditions (Lambers et al., 2012; Lynch, 2011). The rhizosphere itself functions as a highly dynamic interface extending from the rhizoplane into bulk soil over micrometer- to millimeter-scale distances, within which P transformation processes are mediated by both root- and microbially-derived organic acids and phosphatases (Klein et al., 2022). Simultaneously visualizing both enzymes at fine spatial resolution therefore offers a more complete picture of how P mobilization pathways shift as soil pH rises. However, conventional enzyme assays rely on destructive sampling and measure only potential activity, failing to capture actual enzymatic dynamics (Burns et al., 2013; Nannipieri et al., 2012). *In situ* soil zymography has recently emerged as an effective, high-resolution, nondestructive method for visualizing multiple enzyme activities simultaneously, enabling direct observation of rhizosphere processes under near-natural conditions (Albrecht et al., 2025; Ma et al., 2018a; Jia et al., 2024).

Previous research on red soil amelioration and P limitation in *C. oleifera* systems has

focused primarily on bulk soil physicochemical properties and general shifts in enzyme activity or microbial community composition (Li et al., 2020; Wu et al., 2021; F. Wu et al., 2025). Although recent advances integrating in situ enzyme visualization, pH mapping, and root morphological analysis have advanced our understanding of rhizosphere P acquisition dynamics in herbaceous crops and temperate woody species (Ma et al., 2018a; Gillini et al., 2025; Kaloterakis et al., 2024; Jia et al., 2024), their application to perennial woody oil crops cultivated in strongly acidic tropical and subtropical Ultisols remains limited. Given the architecturally complex root system of *C. oleifera* and its specific environmental adaptations, its rhizosphere P dynamics under combined soil acidification and amendment application likely differ substantially from those reported in previously studied systems. Spatially explicit mapping of P transformation and enzyme activity at the root–soil interface is therefore needed to reveal which rhizosphere microdomains govern P acquisition once the plant's native foraging strategy is altered by human intervention, providing a mechanistic basis for optimizing fertilization in cultivation systems characterized by pronounced alternate bearing.

This study represents an initial effort to evaluate the remediation efficacy of combining novel amendments with P application in acidified red soils, together with the associated P acquisition strategies of *C. oleifera*. Three hypotheses were proposed: 1) amendments would alleviate soil acidity and reduce root lignification, potentially shifting the plant's adaptive traits toward spatial soil exploration; 2) this pH amelioration would alter the relative contributions of plant and microbial-derived phosphatases, resulting in distinct rhizosphere enzymatic distribution patterns; 3) the combined application of

amendments and P would synergistically enhance P bioavailability by coupling physical root foraging with biochemical rhizosphere activation. To test these hypotheses, a controlled microecological remediation experiment was conducted using an indoor rhizobox system. This approach integrated *in situ* two-dimensional soil zymography with root morphological analysis in *C. oleifera* cultivated in acidic red soil. The objectives were to evaluate the effects of novel amendments on soil acidification and plant growth, and to characterize the spatial distribution of rhizosphere phosphatase activity under combined amendment and P treatments at the microscale, elucidating the mechanisms underlying synergistic P mobilization.

Materials and methods

Study site and Soil amendment preparation

The soil used in this study was collected from a 16-year-old *C. oleifera* plantation located in Lengshuitan District, Yongzhou City, Hunan Province, China (111°30'57" E, 26°25'12" N). The region has a subtropical monsoon climate, with a mean annual temperature of 17.6 °C. The soil, derived from Quaternary red clay, is classified as an Ultisol according to the United States Department of Agriculture soil taxonomy (Soil Survey Staff, 2014). Fresh surface soil samples (0 - 20 cm) were collected and homogenized. After transport to the laboratory, stones, plant residues, and visible roots were removed manually. The soil was then air-dried at ambient temperature and sieved through a 2 mm mesh for subsequent use in rhizobox experiments. The baseline soil properties were as follows: pH 4.5 (soil: water ratio of 1:2.5), soil organic matter (SOM)

3.92 g kg⁻¹, total nitrogen (TN) 24.32 g kg⁻¹, total P 0.24 g kg⁻¹, total potassium (TK) 18.72 g kg⁻¹, available phosphorus (AP) 3.39 mg kg⁻¹, available potassium 70.33 mg kg⁻¹, ammonium nitrogen 8.30 mg kg⁻¹, and nitrate nitrogen 8.72 mg kg⁻¹ (Liang et al., 2020).

The amendment applied in this study was provided by Guangxi Diyuanzhiben Biological Technology Co., Ltd. (Nanning, China). It consisted mainly of bone meal, silkworm frass, calcium–magnesium phosphate fertilizer, potassium fulvate, and organic manure. The physicochemical properties of the amendment included a total nutrient content of 6.59%, pH 7.88, TN 15.20 g kg⁻¹, TK 26.30 g kg⁻¹, AP 24.40 mg kg⁻¹, and total organic matter 23.66% (Fan et al., 2026).

Experimental setup

The rhizobox experiment was conducted from April to July 2025 at the Key Laboratory of Economic Forestry, Central South University of Forestry and Technology (Hunan, China). Soil sample (3.50 kg) was thoroughly mixed with varying gradients of amendment levels and packed into specially constructed rhizoboxes (40 cm in height × 25 cm in length × 4 cm in width). One side of each rhizobox was detachable, and the soil bulk density was adjusted to 1.5 g cm⁻³ (Supplementary Fig. S1). Uniform two-year-old *C. oleifera* seedlings were selected and transplanted into the rhizoboxes, with two seedlings per box and four replicates per treatment. Soil moisture was maintained at 60% of field capacity by daily watering. The rhizoboxes were placed at a 45° inclination toward the planted side to facilitate root growth along the observation surface. After a 7-day acclimation period, the experimental treatments were initiated. A volume of 200 mL of P

solution was applied at 7-day intervals. The experiment followed a factorial design with three amendment levels (0, 60, and 120 g per rhizobox, designated as T0, T1, and T2) and three P concentrations (0.005 mmol L⁻¹, 0.5 mmol L⁻¹, and 1 mmol L⁻¹ KH₂PO₄, designated as P1, P2, and P3) (Simarani et al., 2018). This design resulted in nine treatment combinations with a total of 36 rhizoboxes (Cano-Ramírez et al., 2017).

Plant sample analysis

At 60 days after treatment initiation, soil zymography was first conducted on intact rhizoboxes prior to plant harvest, so that enzymatic activity measurements were obtained under undisturbed root soil conditions. Following zymography, rhizoboxes were opened and plants were carefully excavated for morphological and physiological analysis, and rhizosphere soil samples were collected for physicochemical characterization. Three *C. oleifera* seedlings were randomly selected for evaluation of morphological and physiological traits. Seedling height was measured using a tape measure, and basal diameter was recorded with a vernier caliper. After careful excavation, intact plants were washed under running water and gently blotted dry with filter paper before being transported to the laboratory. Belowground root systems were scanned with an Epson Expression 11000XL scanner to obtain high-resolution digital images. These images were analyzed with WinRHIZO software (Regent Instruments, Sainte-Foy–Sillery–Cap-Rouge, Quebec, Canada) to determine total root length, root surface area, number of root tips, forks, and crossings. Aboveground and belowground tissues were digested with sulfuric acid and hydrogen peroxide, and total P concentrations were determined with a Smartchem 200 continuous-flow analyzer (Westco Scientific Instruments, Rome, Italy). Root vitality was

assessed using the triphenyltetrazolium chloride (TTC, $C_{19}H_{15}ClN_4$) reduction method described by Yi et al., (2007). Root lignin staining and visualization were performed as described by (Fu et al., 2014), and lignin content was quantified using a commercial assay kit (Solarbio, Beijing, China) according to the manufacturer's instructions. Stained root sections were observed and photographed at 100 $\times$ magnification under an optical microscope (Olympus BX53, Olympus Corporation, Tokyo, Japan).

Soil sample analysis

Soil pH was measured at a soil: water ratio of 1:2.5 using a PHS-3C pH meter (Shanghai INESA Scientific Instrument Co., Ltd., China). Cation exchange capacity (CEC) was determined by ammonium acetate (CH_3COONH_4) extraction (Abdel-Fattah et al., 2015). Soil organic carbon (SOC) was measured using the potassium dichromate oxidation method, and SOM was calculated by multiplying SOC by a factor of 1.724 (Zhou et al., 2025). Total P and AP were determined using the molybdenum–antimony colorimetric method (Sparks et al., 1996). TN was measured according to established Chinese forestry standards (Page, 1982). Ammonium nitrogen and nitrate nitrogen were extracted with 2 mol L⁻¹ potassium chloride and quantified using a continuous flow analyzer (Sims, 1989). TK was measured using a Model 410 flame spectrophotometer (Kirk, 2002; Li et al., 2020). Microbial biomass phosphorus (MBP) was determined by the chloroform fumigation method (Vance et al., 1987). Biologically based phosphorus (BBP) fractions were sequentially extracted using 0.01 mol L⁻¹ calcium chloride ($CaCl_2$), 0.01 mol L⁻¹ citric acid ($C_6H_8O_7$), 0.02 EU L⁻¹ phosphatase, and 1 mol L⁻¹ hydrochloric acid (HCl). The concentrations of individual P fractions ($CaCl_2$ -P, citrate-P, enzyme-P, and HCl-P) were

quantified using the malachite green method (Ohno and Zibilske, 1991) with a SpectraMax i3 multimode microplate reader (Molecular Devices, USA).

Soil zymography and imaging procedures

Rhizosphere phosphatase activities were assessed at 60 days using *in situ* zymography with 4-methylumbelliferone (MUF) as a fluorescent indicator (Razavi et al., 2019). The substrate solution contained 12 nmol L⁻¹ 4-methylumbelliferyl phosphate (MUF-P) dissolved in 10 mmol L⁻¹ MES buffer (C₆H₁₃NO₄SNa), adjusted to pH 6.5 for acid phosphatase (ACP) and pH 11.0 for alkaline phosphatase (ALP). Rhizoboxes were opened from the root observation side, and saturated polyamide membrane filters (0.45 µm pore size, 10.0 cm × 10.0 cm; Taoyuan, China) were placed directly onto the exposed root–soil surface. Membranes were covered with aluminum foil, pressed with a black plate, and incubated in the dark for 1 h. After incubation, membranes were carefully removed, cleaned to remove adhering soil particles, and subjected to fluorescence analysis for ACP and ALP activity (Albrecht et al., 2025; Wang et al., 2023). Zymography was performed first for ACP, then for ALP, with freshly prepared membranes used independently for each enzyme assay to prevent cross-contamination between substrate solutions (Xie et al., 2025). Fluorescence images were captured using a Canon R100 camera under 355 nm ultraviolet illumination with fixed imaging conditions. A calibration curve was prepared by imaging 2 cm × 2 cm membrane sections exposed to a gradient of MUF concentrations (0, 0.1, 0.2, 0.5, 1, 2, 4, 6, and 10 mM).

Image processing and analysis

Zymography images were analyzed using ImageJ software (Wayne Rasband and contributors, National Institutes of Health, USA). The workflow included four main steps: conversion of fluorescence signals to grayscale values, background correction, root segmentation with skeletonization and radius measurement, and transformation of grayscale values into enzymatic activity values (Ma et al., 2018a). Images were first converted to 8-bit grayscale format, followed by correction for illumination variation and camera noise (Kaloterakis et al., 2024). After determining the soil background value, grayscale intensities were converted into enzymatic activity using the calibration curve. Zymograms were then rendered as pseudocolor images using lookup tables (LUTs) to improve visualization. Regions with high signal intensity (red) indicated enzymatic activity hotspots, whereas low-intensity regions (blue) represented areas with limited activity (Jia et al., 2024). Hotspots were defined as regions where enzymatic activity exceeded the mean value by 50% or reached a grayscale threshold of 150% (Heitkötter and Marschner, 2018). Pixel-based enzymatic activity within hotspot regions was calculated by subtracting the background value at zero concentration from the mean grayscale value of three replicates (Ma et al., 2018b). The hotspot percentage was expressed as the ratio of hotspot pixel area to the total image area.

To assess the radial distribution of enzymatic activity relative to root distance, the procedure described by Wei et al., (2019) was followed. Clearly identifiable primary roots were selected, and a distance of 0.2 mm from the root surface was defined as the rhizosphere boundary. The Plot Profile function in ImageJ was used to obtain activity gradients as a function of distance from the root center, which were fitted using a sigmoid

254 Hill equation (Eq. 1):

$$255 \quad V = \min + \frac{\max - \min}{1 + (x/EC)^{-Hillslope}} \quad (1)$$

256 where V represents enzymatic activity, x denotes the independent variable (distance
257 from the root center), and $\min$ and $\max$ correspond to the lower and upper asymptotes of
258 the fitted curve, indicating minimum and maximum enzymatic activity, respectively. EC
259 represents the inflection point of the curve, and $Hillslope$ describes the steepness of the
260 curve at this point.

261 **Statistical analysis**

262 Statistical analyses of soil physical and chemical properties and *C. oleifera* responses
263 under different amendment and P treatments were conducted using IBM SPSS Statistics
264 25.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by
265 Duncan's multiple-range test was used to evaluate differences among treatments at the $p <$
266 0.05 significance level. Data in charts and tables are presented as mean $\pm$ standard deviation
267 ($n = 3$). To clarify the relationships between enzymatic activities, amendment application
268 rates, and plant growth parameters, principal component analysis (PCA) was executed
269 utilizing the vegan package in R (version 4.4.3). Furthermore, a partial least squares path
270 model (PLS-PM) was developed to elucidate the potential pathways through which soil
271 amendments and P fertilizers regulate *C. oelifera* growth by modulating soil nutrients,
272 biologically based P, root architecture, and the spatial distribution of enzymatic activity.
273 This model was constructed utilizing the innerplot function within the plspm package in R.
274 Graphical representations were prepared using OriginPro 2024 (OriginLab Corporation,
275 Northampton, MA, USA).

Results

Response of soil properties and *C. oleifera* growth to amendment application

The combined application of soil amendments and P fertilizers alleviated acidification barriers in red soil and modified the rhizosphere physicochemical environment, resulting in significant improvements in the growth and development of *C. oleifera* (Fig. 1; $p < 0.05$). Soil amendment application ameliorated soil acidity, increasing soil pH from 4.09 in the T0 control to 6.76 and 7.09 in the T1 and T2 treatments, respectively (Supplementary Table S2). Alongside this neutralization, the optimized treatments significantly increased CEC (up to 15.75 cmol kg⁻¹) and enriched SOM to a maximum of 12.38 g kg⁻¹ (3.25 times that of the T0P0 control), creating a highly conducive edaphic environment. Under these improved conditions, aboveground growth parameters increased significantly, particularly under the T1P3 and T2P2 treatments. Maximum seedling height (57.03 mm; Fig. 1a), basal diameter (4.86 mm; Fig. 1b), and total biomass (4.19 g and 3.92 g; Fig. 1c, d) were observed in these treatments. Root system architecture was also significantly altered by the combined treatments, with increases in total root length, surface area, and branch numbers (Fig. 2; Table S1). The T2P2 treatment resulted in the highest total root length, representing a 216.98% increase compared with T0P0. Root branching was most pronounced under the T1P3 treatment, where the number of root forks reached 1135.67 ± 64.09 per plant, corresponding to a 144.23% increase relative to the T0P0 control. Physiological activity of roots was also significantly enhanced (Fig. 2b, c; $p < 0.05$). Root vitality reached its highest value under the T2P3 treatment (265.46 $\mu\text{g TTF g}^{-1} \text{ h}^{-1}$). Root lignin accumulation decreased from 27.55% in T0P0 to 11.70% in T1P3. These results were consistent with

differences observed in histochemical lignin staining intensity (Fig. 2a; $p < 0.05$).

Concentrations of P in *C. oleifera* and P fractions in soils

The combined application of soil amendments and P significantly altered the distribution of P fractions in red soil ($p < 0.05$; Fig. 3). With increasing amendment and P levels, AP reached a maximum value of 94.41 mg kg⁻¹ under the T0P3 treatment, which was 12.68% higher than that observed under T1P2 (Fig. 3a). MBP also increased with increasing gradients of amendment and P levels. Within each amendment level, MBP under P3 was higher than under P0 by 159.0%, 149.0%, and 270.0% for T0, T1, and T2, respectively, whereas differences among amendment treatments at P0 were limited (Fig. 3b). Among BBP fractions, HCl-P was the dominant component, reaching values between 96.48 and 225.18 mg kg⁻¹ under P3, corresponding to increases of 73.0%–226.0% relative to P0 (Fig. 3c). Enzyme-P and citrate-P were the next most abundant fractions, with enzyme-P reaching a maximum of 85.10 mg kg⁻¹ under the T1P2 treatment. CaCl₂-P represented the smallest fraction and showed a significant difference only under the T2 treatment.

Soil phosphatase activity

The combined application of soil amendments and P fertilizers promoted the spatial expansion of rhizosphere phosphatase activity, with a pattern dominated by ACP (Figs. 4 and 5). After 60 days of treatment, the root systems of *C. oleifera* seedlings exhibited newly formed fine roots that were translucent and milky white. Root tip epidermal cells protruded outward, forming dense root hairs that are clearly visible to the naked eye and closely

adhered to surrounding soil particles (Supplementary Fig. S3). Amendment treatments significantly increased rhizosphere phosphatase activity, with both activity intensity and hotspot proportion of ACP higher than those of ALP (Figs. 4 and 5). Compared with the T0 control, ACP activity under T1 and T2 treatments increased by 424.78%, 71.20%, 207.10%, 244.73%, 92.45%, and 247.28%, depending on P level (Fig. 6). ALP activity showed significant increases only under the T1 treatment, with values higher than the control by 231.0%, 54.07%, and 64.94% ($p < 0.05$; Fig. 6). Within the T0 group, ACP activity remained higher than ALP activity across all P levels, with differences ranging from 8.73% to 67.16%. These results indicate that, in acidic red soils, phosphatase activity hotspots are closely associated with root distribution, with ACP playing a dominant role, particularly in root tip regions.

Extent of enzyme activities in the rhizosphere soil

The combined application of soil amendments and P fertilizers significantly reshaped the radial distribution patterns of rhizosphere phosphatase activity. These spatial patterns were described using the Hill equation (Fig. 7). Across all treatments, maximum phosphatase activity occurred at the root surface and decreased progressively with increasing distance from the root. In the T0 treatment, the inflection points for ACP activity were 0.60, 0.40, and 0.45 mm for P1, P2, and P3, respectively, whereas those for ALP were 0.75, 1.10, and 0.90 mm. Significant differences in the rhizosphere extent of phosphatase activity were observed ($p < 0.05$). The extent of ACP activity reached 1.27 ± 0.15 mm under T1P3 and 1.41 ± 0.13 mm under T2P3. Corresponding values for ALP were 1.34 ± 0.22 mm and 1.49 ± 0.13 mm, respectively. Compared with T2P3, both ACP and ALP activities under T1P3

declined more sharply with increasing distance from the root center, as reflected by Hillslope values of 2.08 and 1.88, respectively. These results indicate that amendment and P application not only increased enzyme activity but also altered its spatial distribution within the rhizosphere.

The response of *C. oleifera* to variations in enzymatic hotspot areas

The PLS-PM model elucidated the key mechanistic pathways within the soil-plant system under combined amendments and P fertilizers. Results demonstrated that the integrated application primarily drove *C. oleifera* biomass accumulation by enhancing the soil nutrient pool. Furthermore, soil biologically based P was identified as the key factor regulating the spatial distribution of enzymatic activity hotspots (Fig. 8, GoF = 0.73). The model indicated that the path relationships effectively explained the interactions among variables, accounting for 97%, 90%, and 91% of the variance in soil nutrients, plant nutrients, and plant biomass, respectively. Notably, soil nutrients and plant nutrients significantly promoted plant biomass accumulation, with path coefficients of 0.72 and 0.33, respectively (Fig. 8a). The combined application of amendments and P, soil nutrients, soil biologically based P, root architecture, and plant nutrients all exhibited significant positive total effects on enzymatic activity hotspots and plant biomass ($p < 0.05$). Specifically, the total effects of the combined amendment and P application and soil nutrients on plant biomass were 94.11% and 95.43%, respectively, suggesting that the modification of soil nutrients by these treatments is the core driver for promoting *C. oleifera* growth. Furthermore, the total effect of soil biologically based P on enzymatic activity hotspots reached 85.40% (Fig. 8b), identifying soil biologically based P as the primary driver

influencing enzymatic activity.

Discussion

Synergistic promotion of root traits and physiological metabolism by combined treatments

The combined application of novel amendments and P fertilizers fundamentally transforms the red soil microenvironment, driving a physiological shift in *C. oleifera* from defense-oriented lignification to active root expansion and enzymatic P mobilization. Specifically, our data reveal that the T1P3 and T2P2 treatments significantly increased soil pH from 4.09 to 6.46 - 7.09 and raised the CEC to 15.75 cmol kg⁻¹ (Supplementary Table S2). The substantial pH increase (approximately 3 units) under the T2 treatment is directly attributable to the alkaline nature of the amendment (pH 7.88), which comprises calcium-magnesium phosphate fertilizer, bone meal, and organic manure. These components supply OH⁻ equivalents and Ca²⁺ ions that neutralize soil acidity through proton consumption and the displacement of exchangeable Al³⁺ from cation exchange sites. Furthermore, the calcium component precipitates soluble Al as Al(OH)₃, effectively removing proton-generating Al hydrolysis. By neutralizing severe acid stress, these treatments established a chemically optimized rhizosphere microenvironment that directly triggered biomass accumulation (Fig. 1) and created highly favorable conditions for nutrient activation (Bouray et al., 2021; Lambers et al., 2012). This fundamental chemical amelioration served as the primary catalyst for reshaping the root system architecture of *C. oleifera*. In the unamended control (T0), roots exhibited a stunted, thickened morphology indicative of

384 severe developmental restriction. However, our root scanning data demonstrate that
385 amendment applications unlocked massive root proliferation, highlighted by a 144.23%
386 increase in root forks under T1P3 (Supplementary Table S1). Crucially, our *in situ*
387 zymography visually confirmed that this structural adjustment significantly expanded the
388 soil area explored by the roots, directly corresponding to a broader distribution of enzymatic
389 activity hotspots (Supplementary Fig. S2; Table S1), fully supporting our first hypothesis
390 (Gillini et al., 2025; Ma et al., 2018a). By physically extending the boundaries of the root –
391 soil interface, the plant successfully intercepts previously unreachable nutrient patches
392 within the heterogeneous soil matrix. This provides concrete empirical evidence that
393 structural expansion operates as a highly efficient spatial foraging strategy (Lynch, 2011)
394 to ensure a continuous substrate supply for the expanded enzymatic network (Kuzyakov
395 and Blagodatskaya, 2015). To functionally exploit this massive root network, *C. oleifera*
396 underwent a profound biochemical shift at the micro-scale. Our physiological analyses
397 identified the basis for this enhanced nutrient acquisition, which relies on a critical tradeoff
398 in internal resource allocation. Under the severe acid stress of the T0 treatment, root lignin
399 content reached 27.55% , forming a rigid structural barrier that inadvertently caused P
400 retention within the cell walls (Scherwies et al., 2025). In stark contrast, the amended
401 treatments significantly down-regulated lignification, reducing lignin content to 11.70%
402 under T1P3 while simultaneously increasing overall root vitality (Fig. 2c). This
403 phenomenon indicates that the alleviation of soil acidity eliminates the necessity for
404 structural P retention, safely redirecting internal carbon resources to support metabolically
405 active growth (Albrecht et al., 2025). Ultimately, this reveals a fundamental physiological

transition in *C. oleifera*, shifting from a defense-oriented strategy based on structural P immobilization toward a metabolically active strategy optimized for efficient P utilization (Albrecht et al., 2025).

Activation of soil P fractions and spatial distribution of phosphatase activity in the rhizosphere

The co-application of novel amendments and phosphorus fertilizers fundamentally overcomes P limitation in acidic red soils by synergistically coupling the spatial expansion of rhizosphere enzymatic networks with the chemical activation of recalcitrant P fractions. ACP functioned as a localized mechanism at the root–soil interface, with its activity intensity increasing by up to 424.78% under amendments but its spatial extent remaining confined. In contrast, ALP exhibited a broader spatial distribution, extending into non-rhizosphere regions when MBP was maximized. This spatial acceleration of enzymatic activity facilitated the conversion of HCl-P from a supposedly inert pool into a highly dynamic buffer pool, supplying continuous substrates for enzymatic reactions and supporting P cycling. In acidic, P-deficient red soils, plants primarily rely on phosphatase secretion within the rhizosphere microenvironment to access P (Hinsinger et al., 2011; Wen et al., 2019). Our visual data confirmed that under the T0, T1, and T2 treatments, ACP activity hotspots remained strictly localized along living root surfaces. This precise spatial confinement reflects that ACP synthesis is entirely driven by *C. oleifera* roots (Fig. 4; Chen et al., 2019). Enzymatic activity was confined to a narrow zone (< 1 mm) surrounding the root surface and decreased rapidly with increasing radial distance (Fig. 6a; Ma et al., 2018a). Although the T1 and T2 treatments expanded the area of ACP hotspots with higher P levels

compared to T0, the sharp radial decrease persisted, indicating that the amendments significantly enhanced activity intensity without altering the fundamental spatial confinement of ACP (approximately 1.5–2.0 mm; Fig. 7). Despite this spatial restriction, functional capacity was vastly improved. Higher concentrations of BBP under T2, coupled with intense ACP activity at the rhizoplane, formed an efficient catalytic interface that maximized P acquisition per unit root length, fully supporting our second hypothesis (Wu et al., 2025; Xie et al., 2025).

Crucially, our study captured a spatial decoupling between plant-driven ACP and microbial-derived ALP (Fig. 5). In striking contrast to ACP, ALP exhibited a broader spatial distribution under the combined treatments. This expansion demonstrates how the remediation of acidified soil stimulated microbial communities, establishing a broad P activation network at the *C. oleifera* root boundary (Fig. 7; Zheng et al., 2021). Under low P conditions (P1), soil microorganisms released ALP to enhance P availability, thereby alleviating soil P deficiency (Zhou J et al., 2019). The T2P3 treatment provided optimal carbon and P sources for microbial growth, significantly increasing microbial biomass P (MBP; Supplementary Fig. S3) and promoting the extension of ALP activity well into regions beyond the rhizosphere. Conversely, under the T2P1 and T2P2 treatments, ALP hotspot areas were significantly decreased. This reduction was directly associated with lower MBP levels, which limited enzyme production and prevented the formation of a dense enzymatic network comparable to that of T2P3 (Fig. 3b). Furthermore, lower concentrations of enzyme-P and citrate-extractable P may have reduced the biochemical signaling required for microbial ALP secretion into non-rhizosphere regions, thereby

limiting spatial expansion (Fig. 3c; Wang et al., 2023). These distinct spatial distribution patterns between tightly confined ACP and broadly expanded ALP fully support our second hypothesis regarding the spatial decoupling of rhizosphere enzymatic networks.

The spatial expansion of these enzymatic activity zones accelerated P transformation at the root–soil interface and facilitated the conversion among soil P fractions. Acid-soluble phosphorus (HCl-P) is conventionally considered a relatively unavailable pool in red soils (Li et al., 2023; Wu et al., 2022). However, our chemical fraction analyses overturn this assumption. By raising soil pH and exchangeable cation levels, the amendment application promoted the transformation of P from aluminum-bound to calcium-bound forms. Consequently, HCl-P accumulated to 225.18 mg kg⁻¹ under the T2P3 treatment (Supplementary Table S2; Fig. 3c). Similar results have been reported by Yang et al., (2025), who found that increases in soil pH and calcium availability can shift P toward more accessible forms. Rather than remaining inert, our data demonstrate that this newly formed HCl-P functioned as a dynamic buffer pool (Ch'ng et al., 2014). Enhanced root vitality and stimulated microbial metabolism promoted the localized release of protons and organic acids, facilitating the conversion of HCl-P to citrate-extractable P via dissolution and ligand-exchange processes (Adeleke et al., 2017; Hinsinger, 2001). This rapid transformation supplied continuous substrates for enzymatic reactions and supported robust P cycling in the red soil. The significant accumulation of enzyme-P under T1 indicated suggested limited substrate turnover despite increased substrate availability (Wu et al., 2025). In contrast, under T2P3, the combination of higher P uptake, elevated CaCl₂-P levels, and extensive enzymatic hotspot coverage indicates that the rapid depletion of the enzyme-

P pool was consistent with phosphatase-mediated hydrolysis of organic substrates into bioavailable inorganic forms for *C. oleifera* uptake (Pang et al., 2024).

Regulatory mechanisms of phosphorus uptake strategies based on the PLS-PM

This study quantified the phosphorus acquisition mechanisms of *C. oleifera* in acidic red soil using a PLS-PM. The amendments first eliminated soil acidification through chemical remediation and constructed a BBP buffer pool. Subsequently, high P concentrations induced an explosion of rhizosphere enzymatic activity hotspots, ultimately achieving efficient P transformation and significant biomass accumulation (Fig. 7, GoF = 0.73). These results statistically support our third hypothesis. The model results revealed that the combined application of amendments and P had a strong positive direct effect on soil nutrients (path coefficient 0.98). This indicates that amendment application effectively increased pH, CEC, and SOM content, fundamentally reshaping the basal fertility of the rhizosphere physicochemical environment (Supplementary Table S2) (Zhou et al., 2025). Such improvements in soil quality significantly advanced the spatial expansion of root architecture at a physical level (path coefficient 0.62) and greatly enriched biologically available P (BBP) reserves at a chemical level (path coefficient 0.82). However, the model also identified a high degree of overlap between root morphology and phosphatase activity. PLS-PM analysis showed that the direct positive effect of BBP on enzymatic activity hotspots reached 0.85, whereas the direct path coefficient from root architecture to these hotspots was only -0.05. This strong contrast statistically confirms that in the high fertility state of remediated acidic red soil, the construction of intensive rhizosphere enzymatic reaction centers no longer relies solely on the extensive expansion of root surface area.

Instead, it highly depends on the enzyme labile substrates such as Enzyme-P and Citrate-P (Li et al., 2023; Wu et al., 2025). High BBP concentrations act as potent biochemical signals, directly stimulating roots and microbes to secrete large amounts of phosphatases (Xie et al., 2025). This explains why the T2P3 treatment achieved the highest P acquisition efficiency despite not having the maximum root biomass, as the biochemical efficiency between substrates and enzymes far outweighed the importance of physical contact area. Through this cascade effect, P accumulation and biomass growth in *C. oleifera* were ultimately promoted. Soil nutrients (path coefficient 0.72) and plant nutrients (path coefficient 0.33) together constituted the decisive factors for biomass increase, with an explained variance of 91%. Furthermore, BBP not only indirectly promoted P uptake by regulating enzymatic activity but also provided nutrients to the plants through a direct path (path coefficient 0.30) (Pang et al., 2024).

Conclusion

This study investigated the effects of combined organic amendment and phosphorus fertilization on P acquisition by *Camellia oleifera* in acidic red soils using in situ soil zymography, root morphological analysis, and partial least squares path modeling. Amendment application effectively alleviated soil acidification (pH from 4.09 to 7.09) and improved soil physicochemical properties. These changes were associated with enhanced root development (root length increased by 217%, branching by 144%) and reduced root lignin content (from 27.55% to 11.70%). Phosphatase activity was elevated, with acid phosphatase increasing up to 424.78% at the root surface, accompanied by shifts in soil P fractions from recalcitrant HCl-P toward more labile pools. PLS-PM analysis indicated that

516 soil nutrients were positively associated with both root morphology (path coefficient = 0.62)
517 and bioavailable P pools (0.82), while bioavailable P showed a strong positive link to
518 enzymatic activity (0.85) and directly to plant P uptake (0.30). Among treatments, the
519 combination of amendment T2 with moderate P supply (T2P2) maximized root foraging
520 capacity, while T2 with high P supply (T2P3) sustained the highest enzymatic activity.
521 These findings suggest that organic amendment combined with appropriate P fertilization
522 can improve P utilization efficiency in *C. oleifera* plantations on acidic soils, providing a
523 practical basis for soil management in subtropical forestry systems.

CRedit authorship contribution statement

Huimin Yin: Writing - original draft, Visualization, Formal analysis, Data curation, Investigation. **Yuhua Li:** Writing-review & editing. **Xinyi Zhang:** Methodology, Writing-review & editing. **Shanshan Su:** Methodology. **Yuhong Li:** Methodology, Writing - review & editing. **Lichao Wu:** Writing - review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Xiaojun Jiang:** Writing - review & editing, Supervision, Resources. **Jun Yuan:** Writing - review & editing, Supervision, Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

545 Data will be made available on request.

546 **Supplementary material**

547 Supplementary Figs. S1–S3 and Tables S1–S2 are available online.

548

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759

760 **List of figure captions**

761 **Fig 1. Effects of combined application of soil amendments and phosphorus levels on**
762 **morphological traits and biomass of *Camellia oleifera* seedlings.** (a) Seedling height; (b)
763 stem diameter; (c) shoot dry biomass; (d) root dry biomass; (e) shoot phosphorus content;
764 (f) root phosphorus content. T0, no amendment; T1, 60 g amendment; T2, 120 g
765 amendment; P1, 0.005 mmol L⁻¹ KH₂PO₄; P2, 0.5 mmol L⁻¹ KH₂PO₄; P3, 1 mmol L⁻¹
766 KH₂PO₄. Error bars represent standard deviation. Different lowercase letters indicate
767 significant differences among treatments determined by ANOVA followed by Duncan's
768 post hoc test ($p < 0.05$).

Fig 2. Effects of combined application of soil amendments and phosphorus on root structure, lignin deposition, and metabolic activity in *Camellia oleifera*. (a) Root anatomical structure; (b) root lignin staining; (c) root activity. T0, no amendment; T1, 60 g amendment; T2, 120 g amendment. Error bars represent standard deviation. Different lowercase letters indicate significant differences among treatments determined by ANOVA followed by Duncan's post hoc test ($p < 0.05$).

Fig 3. Phosphorus distribution in *Camellia oleifera* and soil phosphorus fractions under different soil amendment and phosphorus treatments. (a) Available phosphorus; (b) microbial biomass phosphorus; (c) soil phosphorus fractions extracted using CaCl_2 , citrate, enzyme, and HCl. T0, no amendment; T1, 60 g amendment; T2, 120 g amendment. Error bars represent standard deviation ($n = 3$). Different lowercase letters indicate significant differences among treatments determined by ANOVA followed by Duncan's post hoc test ($p < 0.05$).

Fig 4. Zymogram of acid phosphatase activity in the rhizosphere of *Camellia oleifera*. The color maps indicate enzyme activity ($\text{pmol mm}^{-2} \text{h}^{-1}$).

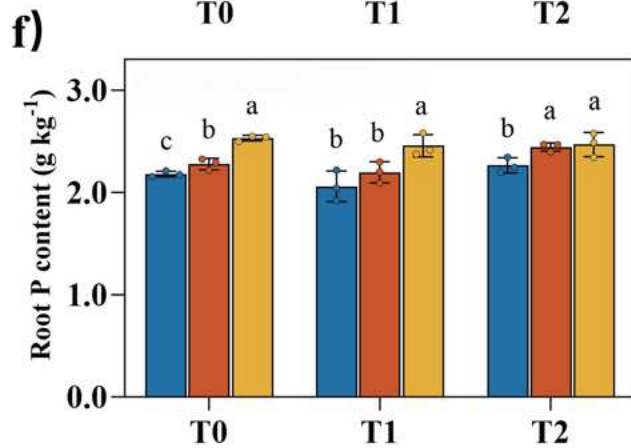
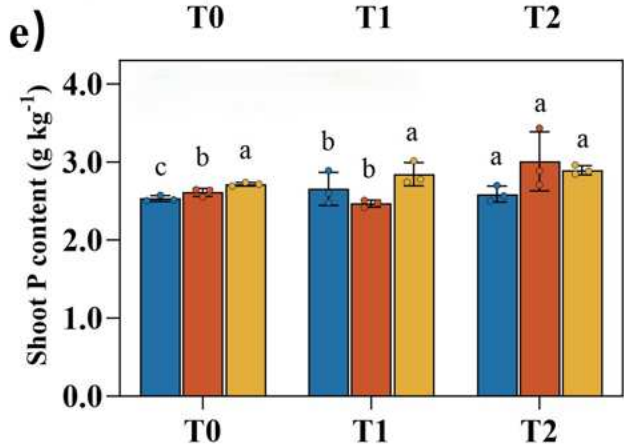
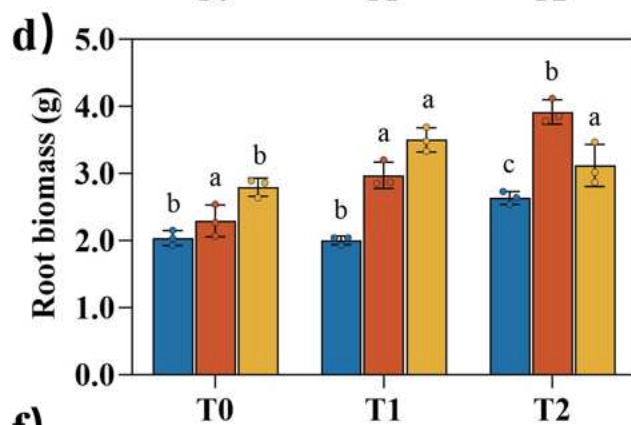
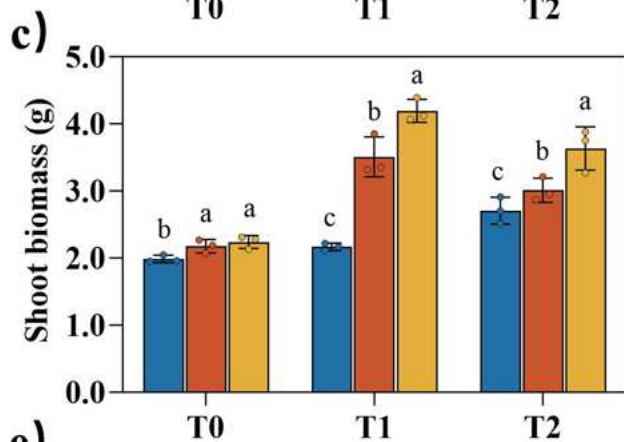
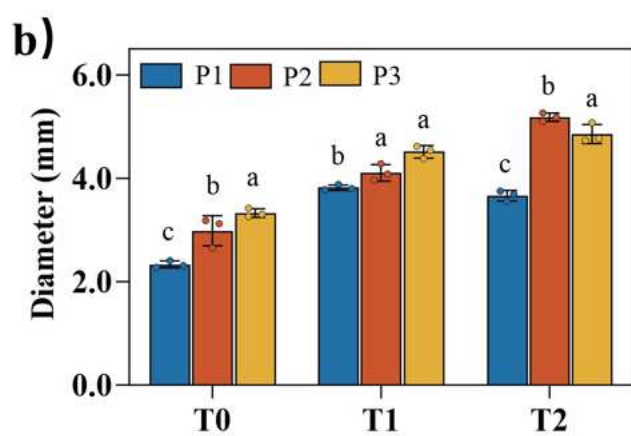
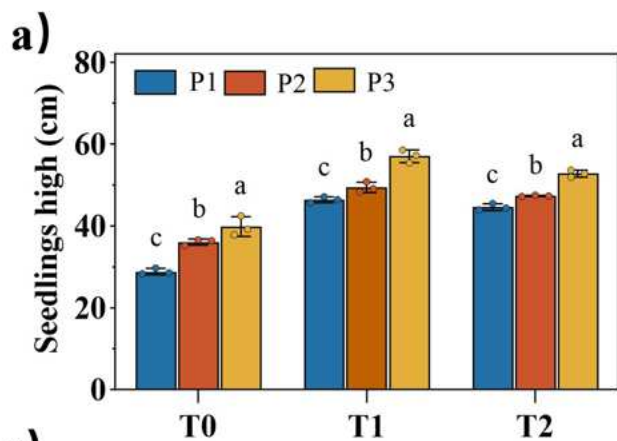
Fig 5. Zymogram of alkaline phosphatase activity in the rhizosphere of *Camellia oleifera*. The color maps indicate enzyme activity ($\text{pmol mm}^{-2} \text{h}^{-1}$).

Fig 6. Proportion of hotspots for acid phosphatase (ACP) and alkaline phosphatase (ALP) activities in the rhizosphere of *Camellia oleifera*. Values are presented as mean $\pm$ standard deviation. Different lowercase letters indicate significant differences among phosphorus levels within the same amendment treatment ($p < 0.05$).

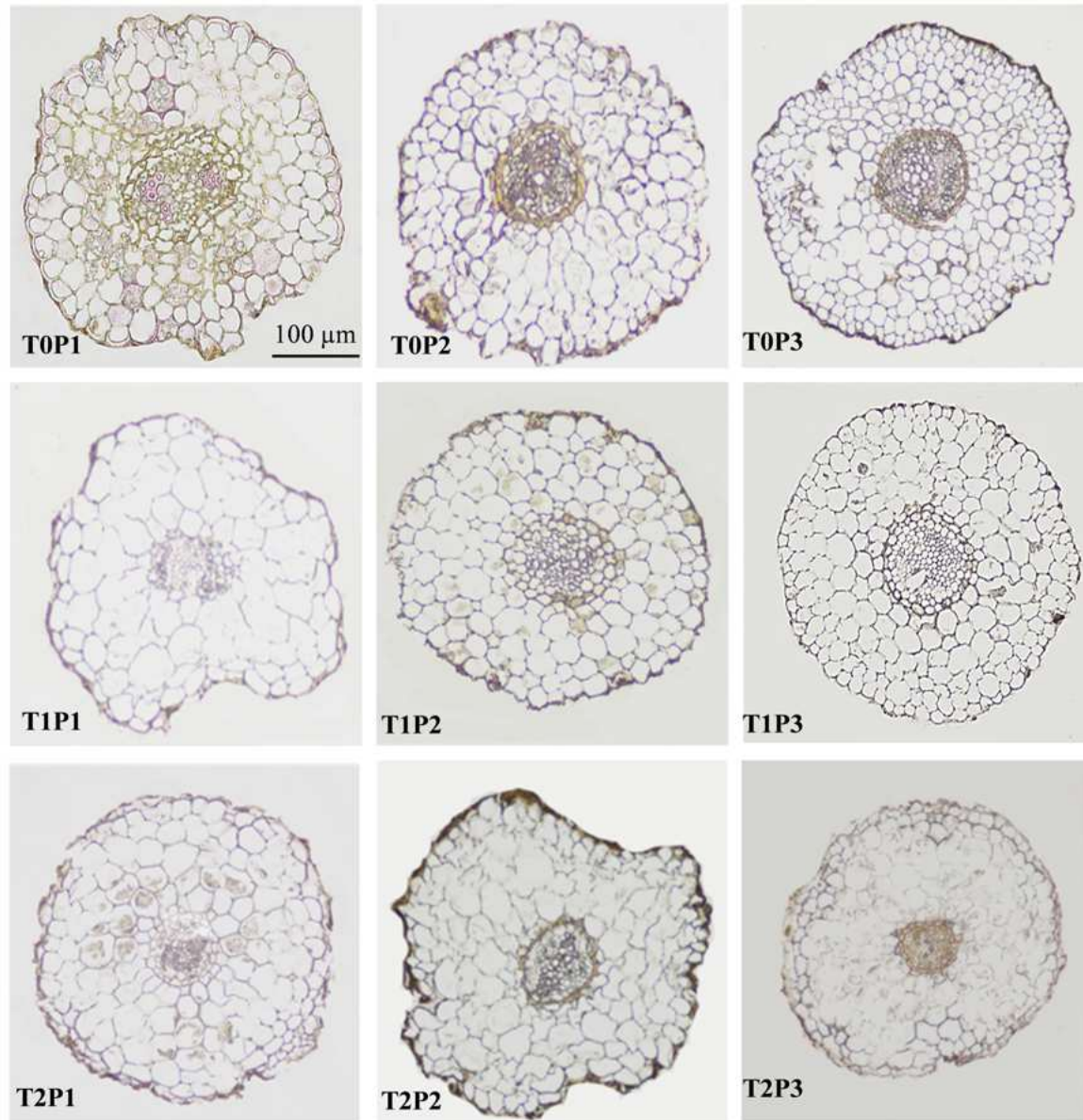
Fig 7. Spatial distribution of enzyme activities in the rhizosphere of *Camellia oleifera*. Curves represent Hill function fits describing enzyme activity as a function of horizontal distance from the root center. (a) T0, no amendment; (b) T1, 60 g amendment; (c) T2, 120 g amendment. Dotted lines indicate acid phosphatase (ACP), and solid lines indicate alkaline phosphatase (ALP). Models are based on mean values from 10 vertical measurement points per radial distance per plant ($n = 3$). Error bars are omitted to improve visualization; standard errors were consistently $<10\%$ of the measured activity values.

Fig. 8 Pathways (a) and total effects (b) identified by partial least squares path modeling (PLS-PM) of different amendments and phosphorus levels on *C. oleifera* biomass. a) Based on PLS-PM, this panel analyzes the direct and indirect effects of soil nutrient levels, soil biologically based phosphorus (BBP), root architecture, plant nutrient content, plant biomass, and hotspot proportion under different amendment treatments and phosphorus levels. Blue and red arrows indicate negative and positive effects, respectively. All arrows represent significant effect sizes ($p < 0.05$). Numbers on the arrows are significant standardized path coefficients. R^2 values indicate the explained variance. Soil nutrient indicators include pH, cation exchange capacity (CEC), soil organic matter (SOM), total phosphorus (TP), total potassium (TK), total nitrogen (TN), nitrate nitrogen (NO_3^- -N), and ammonium nitrogen (NH_4^+ -N); Soil BBP fractions include CaCl_2 -P, citrate-P, enzyme-

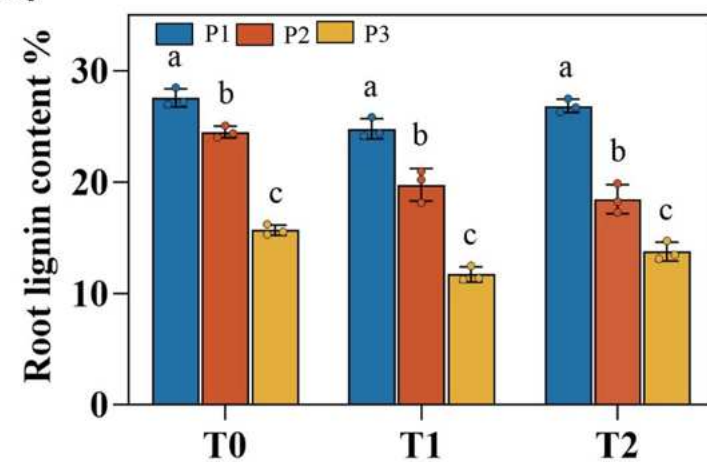
808 P and HCl-P; Root architecture parameters include root length, root surface area, root tips,
809 root forks, and root crossings; Hotspot indicators include activity hotspots of acid
810 phosphatase and alkaline phosphatase; Plant nutrient indicators include shoot phosphorus
811 content and root phosphorus content; Plant biomass indicators include shoot biomass and
812 root biomass; b) This panel analyzes the total effects of soil amendments, phosphorus levels,
813 soil nutrients, BBP, and rhizosphere architecture on plant biomass and enzyme activity
814 hotspots.



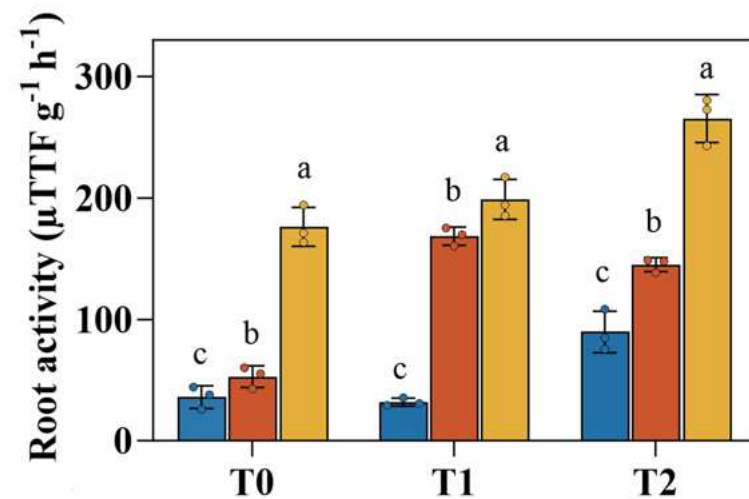
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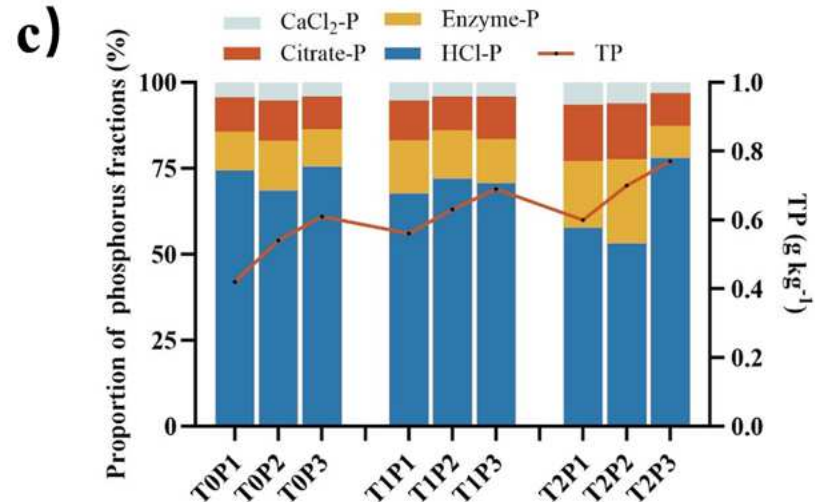
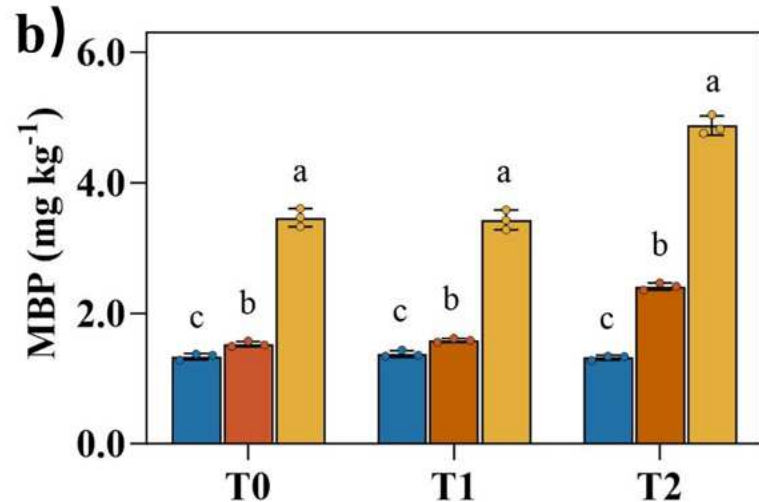
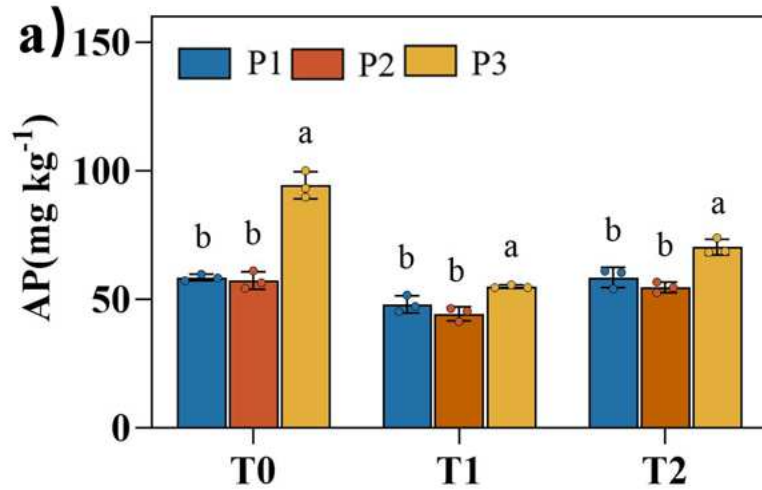


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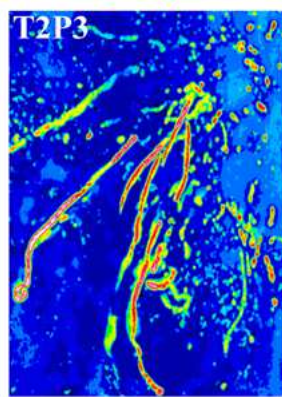
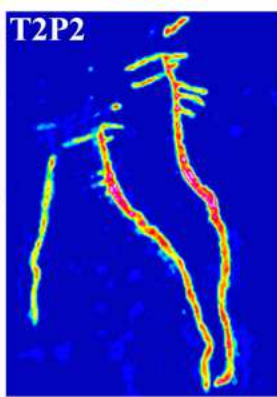
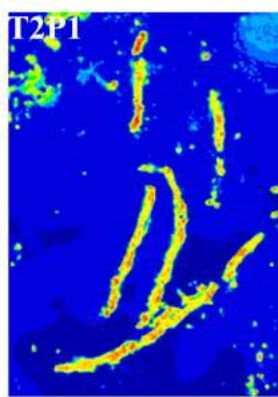
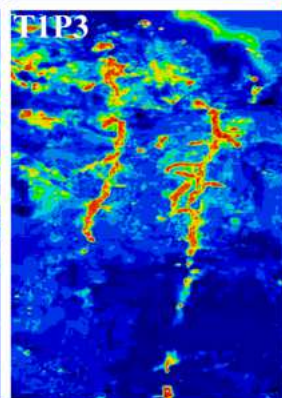
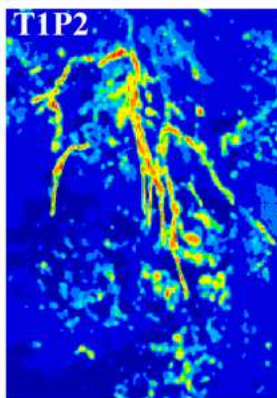
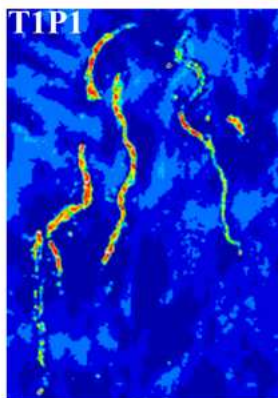
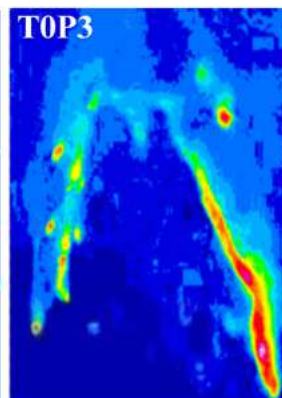
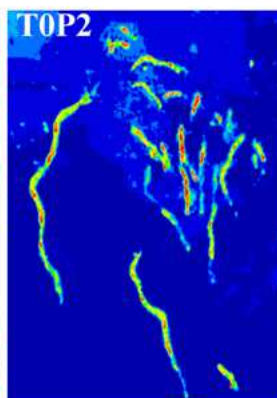
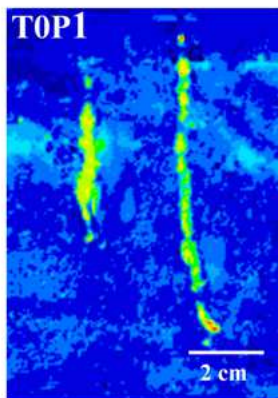
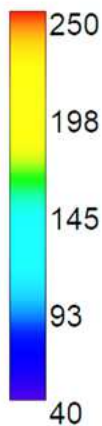


c)

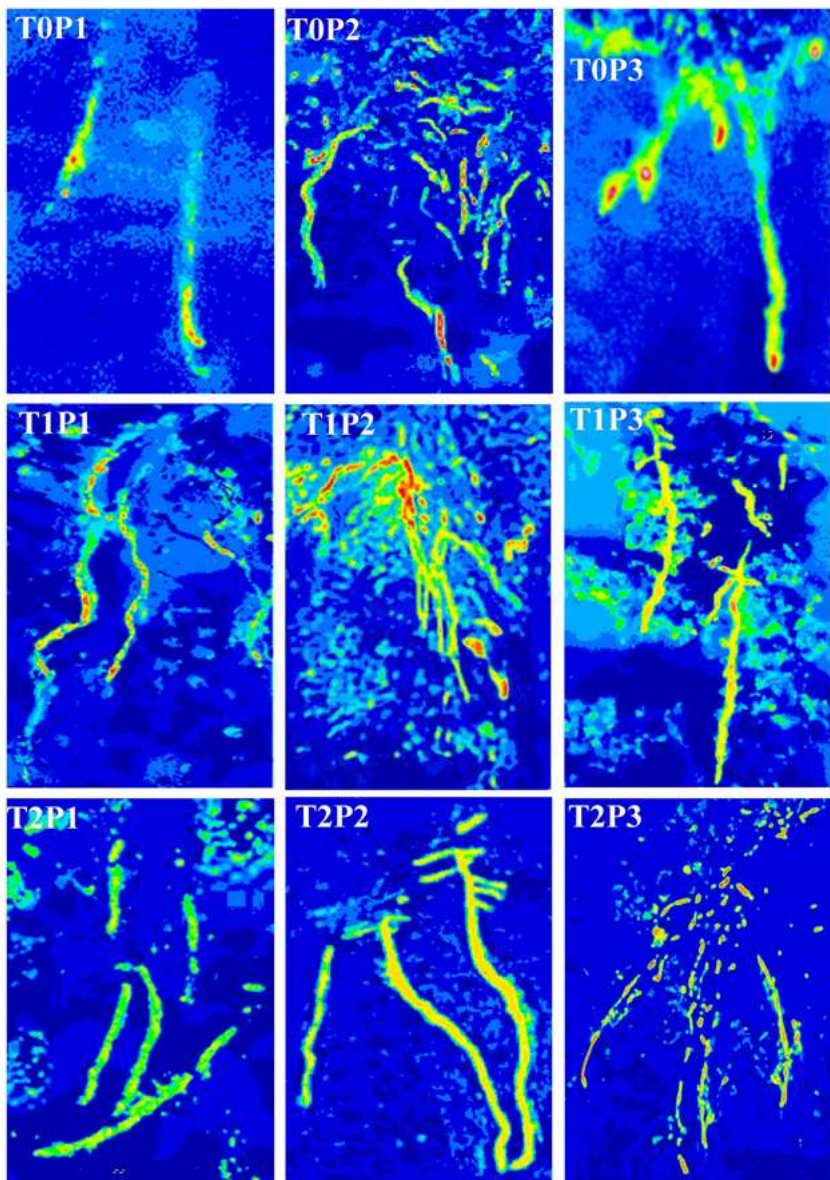
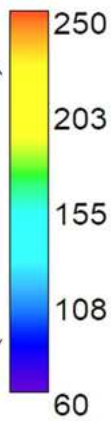


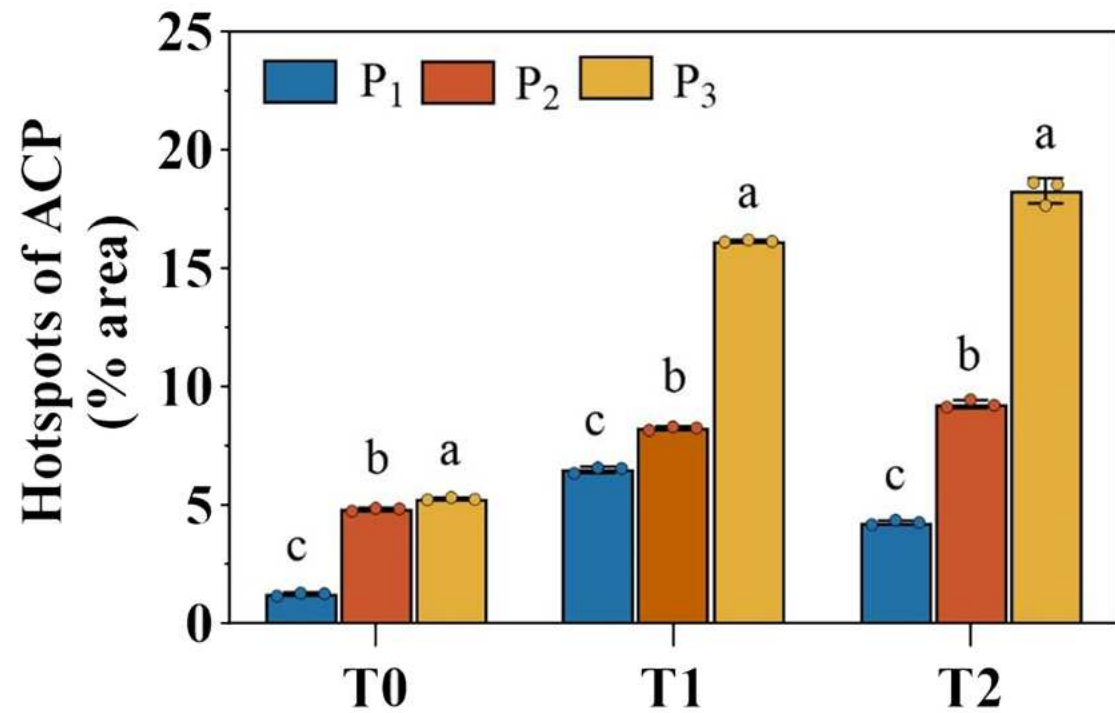
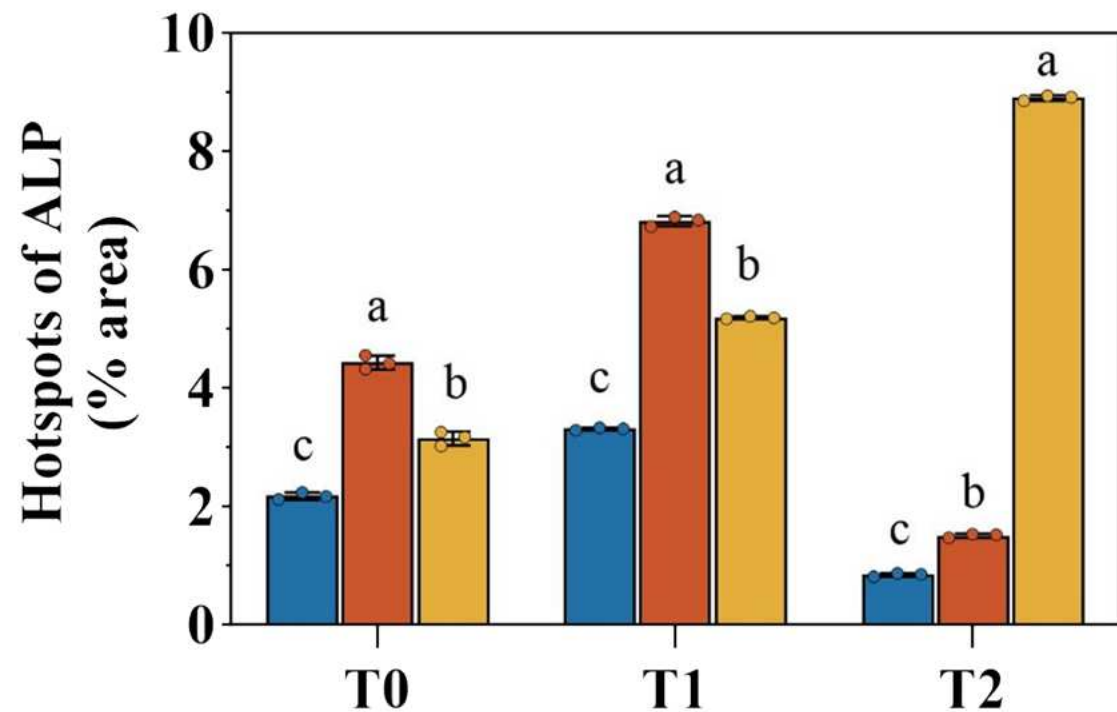


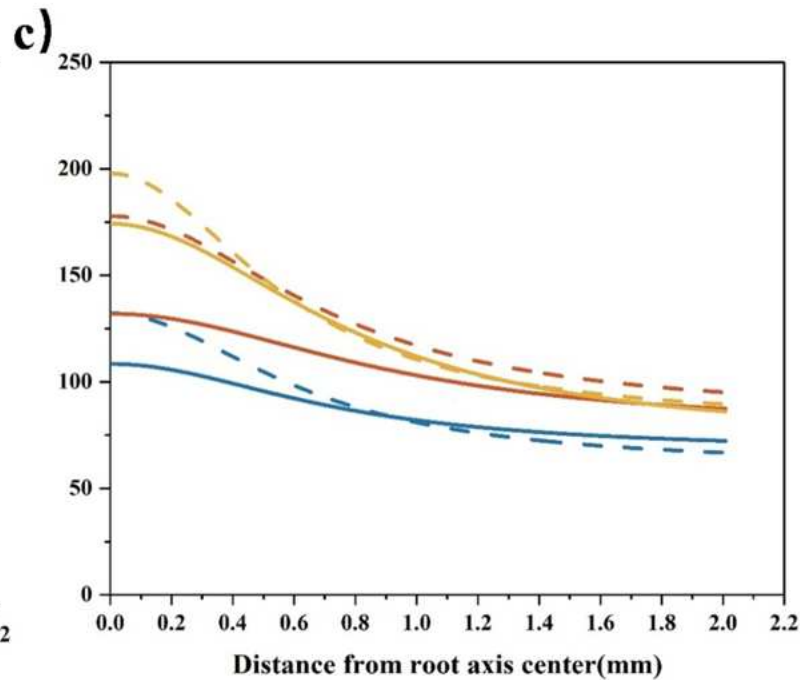
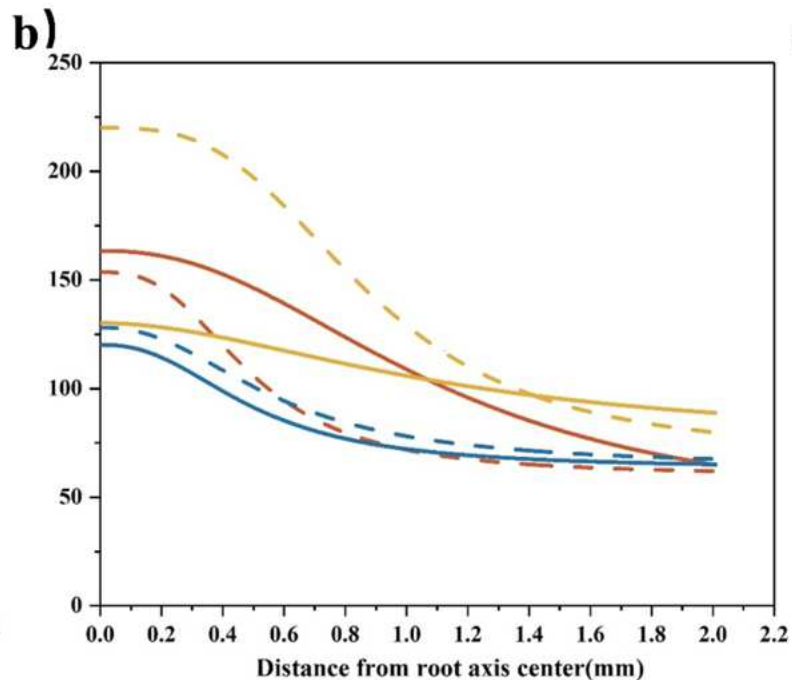
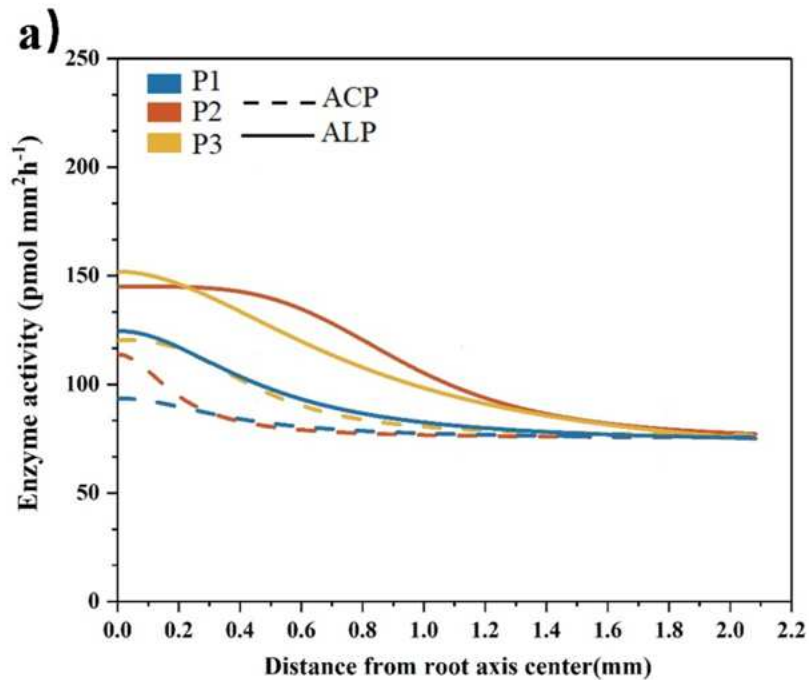
Acid phosphatase
($\text{nmol cm}^{-2} \text{h}^{-1}$)

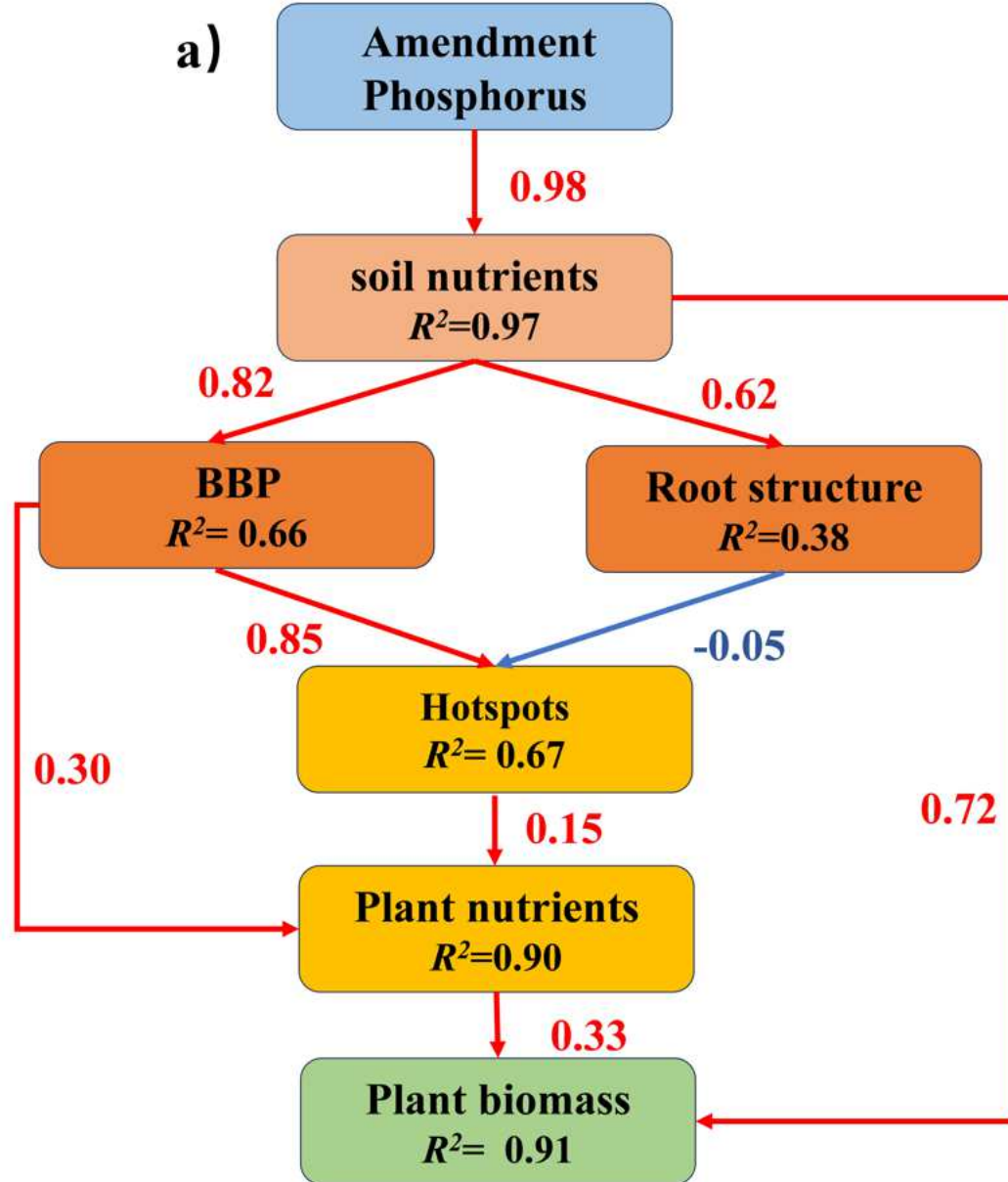


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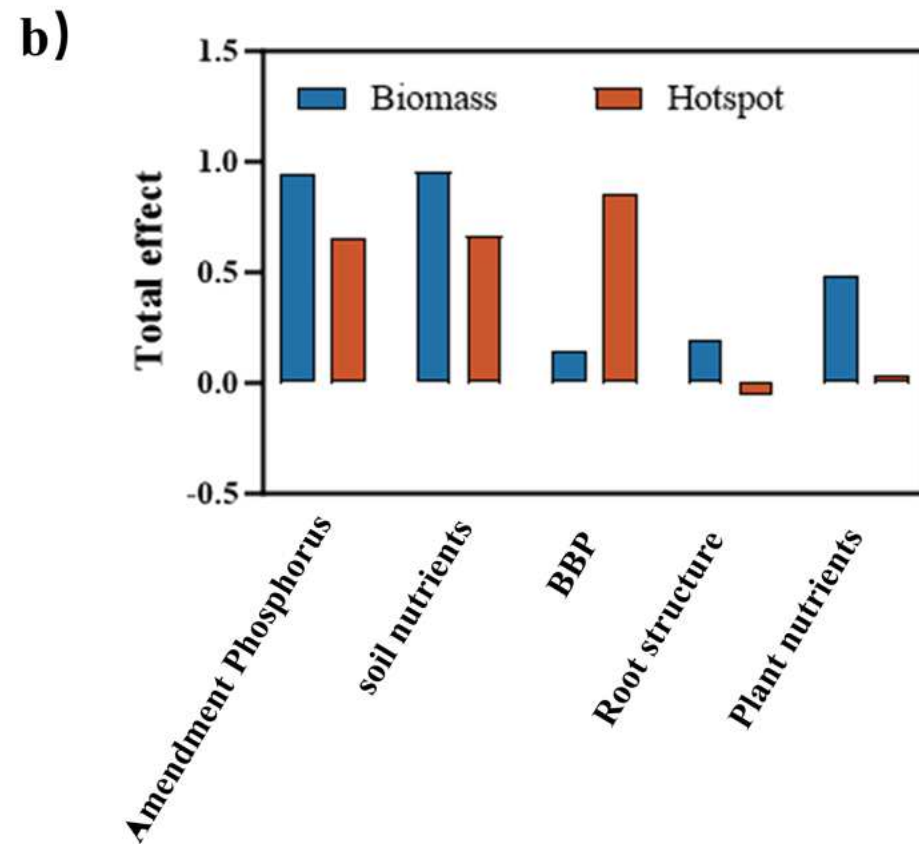


a)**b)**





Goodness of fit =0.73



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