Effects of Converting Chinese Fir Forest to Broad-Leaved Forest on Rhizosphere and Bulk Soil Phosphorus Fractions and Transformation: Postprint

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Abstract

Phosphorus (P) is a key factor in maintaining the productivity of subtropical forest ecosystems. Chinese fir is mainly distributed in subtropical regions of China, where Chinese fir forest soils are acidified with low P utilization efficiency. Investigating the effects of Chinese fir forest conversion on soil P is of great significance for ecosystem stability and sustainable forest management. This study examined replanted Chinese fir forest, Castanopsis hystrix forest, Mytilaria laosensis forest, and Castanopsis hystrix/Mytilaria laosensis mixed forest on clear-cut sites of south subtropical Chinese fir forest, collecting rhizosphere and bulk soils to investigate the effects of converting south subtropical Chinese fir plantation to broad-leaved forest on soil P fractions and transformation. The results showed: (1) The microbial biomass P content and acid phosphatase activity in rhizosphere and bulk soils of the converted Castanopsis hystrix forest, Mytilaria laosensis forest, and Castanopsis hystrix/Mytilaria laosensis mixed forest were significantly higher than those in Chinese fir forest; the total P in Castanopsis hystrix forest and Castanopsis hystrix/Mytilaria laosensis mixed forest was more easily transformed into available P than that in Chinese fir forest and Mytilaria laosensis forest; (2) The calcium chloride-extractable P content in rhizosphere and bulk soils of Castanopsis hystrix forest and Castanopsis hystrix/Mytilaria laosensis mixed forest was significantly higher than that in Chinese fir forest and Mytilaria laosensis forest; the enzyme-extractable P, hydrochloric acid-extractable P, and citric acid-extractable P contents in rhizosphere and bulk soils of Mytilaria laosensis forest and Castanopsis hystrix/Mytilaria laosensis mixed forest were significantly higher than those in Chinese fir forest and Castanopsis hystrix forest; (3) RDA results indicated that the key factors regulating P fractions in rhizosphere and bulk soils were soil water content and microbial biomass carbon, respectively. In summary, converting Chinese fir forest to broad-leaved forest is beneficial for the storage and supply of forest soil P. This study provides important scientific basis for tree species selection and management strategies to improve soil P availability in south subtropical plantations.

Full Text

Preamble

Effects of Conversion of Chinese Fir Forest to Broad-Leaved Forests on Phosphorus Components and Transformation in Rhizosphere and Non-Rhizosphere Soils

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Abstract

Phosphorus (P) is a critical factor maintaining productivity in subtropical forest ecosystems. Chinese fir (*Cunninghamia lanceolata*), widely distributed across subtropical China, is associated with soil acidification and low P use efficiency. Understanding how conversion of Chinese fir plantations affects soil P is essential for ecosystem stability and sustainable forest management. This study examined replanted Chinese fir, *Castanopsis hystrix*, *Mytilaria laosensis*, and mixed *C. hystrix*/*M. laosensis* stands on a Chinese fir clearcut site in south subtropical China, analyzing rhizosphere and non-rhizosphere soils to investigate effects of conversion on soil P fractions and transformation. Results showed: (1) Microbial biomass P content and acid phosphatase activity in rhizosphere and non-rhizosphere soils were significantly higher in converted broad-leaved stands than in Chinese fir. Total P in *C. hystrix* and mixed stands was more readily transformed to available P compared to Chinese fir and *M. laosensis* stands. (2) Calcium chloride-extractable P was significantly higher in *C. hystrix* and mixed stands than in Chinese fir and *M. laosensis* stands, while enzyme-extractable P, hydrochloric acid-extractable P, and citric acid-extractable P were significantly higher in *M. laosensis* and mixed stands. (3) Redundancy analysis identified soil water content and microbial biomass carbon as key regulators of P fractions in rhizosphere and non-rhizosphere soils, respectively. In conclusion, converting Chinese fir plantations to broad-leaved forests enhances soil P storage and supply, providing a scientific basis for tree species selection and management strategies to improve soil P availability in south subtropical plantations.

Keywords: *Cunninghamia lanceolata* plantation; broad-leaved species; rhizosphere soil; phosphorus fraction; south subtropics

Introduction

Phosphorus (P) is an essential nutrient element for plant growth, participating in various physiological activities and cycling continuously between plants and soil. Due to adsorption and fixation by soil colloids, available phosphorus (AP) typically constitutes less than 1% of total phosphorus (TP), making it a primary limiting factor for plant growth. Different tree species exhibit varying capacities for P absorption and utilization, and appropriate plantation conversion can alleviate soil P limitation and improve utilization efficiency. Therefore, exploring how plantation transformation affects soil P is crucial for improving soil nutrients and protecting forest resources.

Soil P fractions are influenced by both biotic and abiotic factors, with their dynamics affecting P availability. Acid phosphatase (ACP) secreted by soil microorganisms and plant roots promotes organic P mineralization and represents a key factor controlling P availability in forest ecosystems. Plantation conversion can alter soil physicochemical properties and microbial activity, thereby influencing P fixation and release. In recent years, plantation conversion has become an important silvicultural measure to address issues of species monotony, biodiversity loss, and soil nutrient depletion in Chinese plantations, with most research focusing on stand age and understory vegetation. However, in subtropical regions, effects of plantation conversion on soil P forms and availability remain poorly understood, and the primary mechanisms driving these changes are unclear. Investigating changes in soil physicochemical properties and microbial activity following conversion, and analyzing associated shifts in P fractions and transformation processes, is essential for improving P bioavailability.

The rhizosphere, defined as the soil zone significantly influenced by root activity, constitutes a critical site for material and energy exchange between soil and plants. Rhizosphere soil provides essential minerals and water for plants and microorganisms, while microbial and root activities reciprocally influence rhizosphere conditions. Following plantation conversion, rhizosphere and non-rhizosphere soils are affected by plant species, litter, and root exudates, leading to differences in pH, nutrient content, and enzyme activity among different species. Generally, rhizosphere soil contains higher nutrient concentrations than bulk soil due to continuous inputs of root exudates, dead root cells, and lysates, resulting in distinct microbial activity patterns.

Chinese fir is widely planted in subtropical China due to its versatile timber and high yield. However, most Chinese fir plantations are pure stands, leading to soil nutrient depletion, biodiversity loss, and declining productivity that severely affect ecosystem stability. Establishing broad-leaved forests represents an effective approach to improve soil properties and slow fertility decline. Compared with conifers, broad-leaved species possess more complex root systems and provide more stable ecosystem services. Specifically, differences in litter, root systems, and exudate chemistry between broad-leaved and coniferous forests drive substantial variation in soil microbial community composition. For instance, *Castanopsis hystrix* and *Mytilaria laosensis* exhibit higher fine root biomass and litter quantity and quality than Chinese fir, promoting greater microbial activity that facilitates P transformation and increases bioavailable P inputs. These species have become preferred broad-leaved timber species in south subtropical China. However, the specific effects of converting Chinese fir plantations to broad-leaved forests on soil P fractions and transformation, and their primary drivers, remain poorly understood—limiting development of sustainable management strategies. This study investigates changes in rhizosphere and non-rhizosphere soil P fractions, microbial biomass P, and ACP activity following conversion of Chinese fir plantations to broad-leaved forests (*C. hystrix*, *M. laosensis*, and mixed stands), identifying key factors influencing soil P fractions to provide a scientific basis for tree species selection and management strategies to enhance soil P availability in south subtropical plantations.

1.1 Study Area Overview

The study site is located in the Fubo Experimental Forest of the Tropical Forestry Experimental Center, Chinese Academy of Forestry, in Pingxiang City, Guangxi (106°39 0 -106°59 30 E, 21°57 47 -22°19 27 N). The region features a subtropical monsoon climate with warm temperatures, abundant heat, and plentiful rainfall with distinct wet and dry seasons. Mean annual temperature is approximately 21 °C, with mean annual precipitation of about 1,400 mm concentrated between April and September. The landscape consists primarily of low mountains and hills with acidic red soils derived from granite weathering under high temperature and alternating wet-dry conditions. Dominant plantation conifers include *Pinus massoniana* and Chinese fir, while major broad-leaved species comprise *Castanopsis hystrix*, *Mytilaria laosensis*, *Michelia macclurei*, *Erythrophleum fordii*, and *Eucalyptus urophylla*, established in both pure and mixed stands.

1.2.1 Sample Plot Establishment

This study selected 31-year-old pure Chinese fir plantations and three broad-leaved forest types (*C. hystrix*, *M. laosensis*, and mixed *C. hystrix*/*M. laosensis*) with similar topography, soil texture, stand age, and management history. Four independent 20 × 20 m plots were randomly established for each forest type, with at least 100 m between plots of the same type. All stands were planted in 1991 on a Chinese fir clearcut site at an initial density of 2,500 trees per hectare, with two thinning operations conducted early in the rotation and no subsequent human disturbance. Within each plot, six 1 m × 1 m nylon mesh collection frames (1 mm aperture) were randomly placed at 0.5 m height to monitor annual litterfall production. Stand characteristics are summarized in .

1.2.3 Sample Collection and Analysis

Soil sampling was conducted in August 2022. Given the random distribution of trees in the mixed *C. hystrix*/*M. laosensis* stands, a systematic grid method was used to determine sampling points. Each plot (20 m × 20 m) was divided into 16 squares of 5 m × 5 m, with sampling points established at grid intersections (9 points per plot). Rhizosphere and non-rhizosphere soil collection followed Cui et al. (2019). After removing surface litter and debris, soils from the 0-10 cm layer were collected. Loosely attached soil was shaken from roots, with remaining root-adherent soil designated as rhizosphere samples and shaken-off soil as non-rhizosphere samples. Samples were sealed, stored at low temperature, and transported to the laboratory immediately. Each soil sample was passed through a 2 mm sieve to remove stones, roots, and fauna. One portion was air-dried and ground for physicochemical analysis, while another was stored at -20 °C for determination of P fractions, enzyme activities, and microbial biomass.

Soil physicochemical properties were determined following methods described in *Soil Agrochemical Analysis* (Bao, 2000). Soil water content (SWC%) was measured by oven-drying; pH was determined using a pH meter (soil:water = 1:2.5, m/V); soil organic carbon (SOC) by potassium dichromate external heating method; total nitrogen (TN) by continuous flow analyzer (SEAL Auto Analyzer3) after H_2SO_4-mixed accelerator digestion; ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) by continuous flow analyzer after extraction with 2 $mol \cdot L^{-1}$ KCl; total P (TP) by H_2SO_4-$HClO_7$ digestion; and available P (AP) by double acid (HCl-H_2SO_4) extraction, both measured by molybdenum blue colorimetry.

Soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were determined by chloroform fumigation-extraction. MBC and MBN were extracted with 0.5 $mol \cdot L^{-1}$ K_2SO_4 and measured on a TOC analyzer (Multi N/C 3100, Germany). MBP was extracted with 0.5 $mol \cdot L^{-1}$ $NaHCO_3$ and measured by molybdenum blue colorimetry.

Soil enzyme activities involved in C, N, and P cycling were measured using 96-well microplate fluorometry. Fresh soil (1.25 g) was suspended in 125 mL CH_3COONa buffer at 4 °C. Two hundred microliters of suspension were pipetted into microplates, supplemented with appropriate substrates, incubated at 25 °C in darkness for 3 h, then reactions were terminated with 5 L of 0.5 $\text{mol} \cdot \text{L}^{-1}$ NaOH. Fluorescence was measured at 365–450 nm using a microplate reader. Enzyme activities were expressed as $\text{nmol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. Enzyme types, functions, and substrates are detailed in .

Soil P fractions were determined using the biologically-based P fractionation method (BBP) of Deluca et al. (2015). Fresh soil (0.5 g) was weighed into four 15 mL centrifuge tubes, to which were added 10 mL of either 0.01 $\text{mol} \cdot \text{L}^{-1}$ CaCl_2, 0.01 $\text{mol} \cdot \text{L}^{-1}$ citric acid, 0.02 $\text{EU} \cdot \text{mL}^{-1}$ enzyme mixture, or 1 $\text{mol} \cdot \text{L}^{-1}$ HCl. Tubes were shaken for 3 h (180 rpm, 25 °C), then 1 mL of supernatant was withdrawn from 2/3 depth, transferred to 1.5 mL tubes, and centrifuged (10,000 rpm, 25 °C) for 1 min. Phosphorus concentration in the supernatant was measured by malachite green method.

1.2.4 Data Analysis

The phosphorus activation coefficient (PAC) was calculated as:

$$\text{PAC} = \frac{\text{AP}}{\text{TP} \times 1000} \times 100\%$$

where AP is available phosphorus ($\text{mg} \cdot \text{kg}^{-1}$) and TP is total phosphorus ($\text{g} \cdot \text{kg}^{-1}$).

Statistical analyses were performed using SPSS 26.0. One-way ANOVA compared differences in soil physicochemical properties, microbial biomass, enzyme activities, and P fractions among stand types for both rhizosphere and non-rhizosphere soils, with least significant difference (LSD) post-hoc tests at $P < 0.05$. Redundancy analysis (RDA) was conducted using Canoco 5, with soil P fractions as response variables and soil physicochemical properties as explanatory variables. Figures were prepared using Origin Pro 2023.

2.1 Physicochemical Characteristics of Rhizosphere and Non-Rhizosphere Soils Following Conversion

As shown in , in both rhizosphere and non-rhizosphere soils, converted *C. hystrix* stands exhibited significantly higher SOC, TN, NH_4^+-N, NO_3^--N, TP, N/P ratio, and SWC compared to Chinese fir stands ($P < 0.05$). Mixed *C. hystrix*/*M. laosensis* stands showed significantly higher SOC, TN, NH_4^+-N, NO_3^--N, AP, C/P ratio, N/P ratio, and SWC than Chinese fir stands ($P < 0.05$). *Mytilaria laosensis* stands displayed significantly higher SOC, TN, NH_4^+-N, NO_3^--N, and SWC than Chinese fir stands ($P < 0.05$).

2.2 Characteristics of Soil Phosphorus Fractions and Activation Coefficients Following Conversion

As illustrated in [Figure 1: see original paper], after conversion from Chinese fir to broad-leaved forests, soil P fractions in both rhizosphere and non-rhizosphere soils followed the pattern: HCl-P > Citrate-P > Enzyme-P > $CaCl_2$-P, with all fractions higher in rhizosphere than non-rhizosphere soils. In rhizosphere soils, $CaCl_2$-P content in *C. hystrix* and mixed stands increased by 33.9% and 21.6% compared to pure Chinese fir stands ($P < 0.05$). Enzyme-P content in *M. laosensis* and mixed stands increased by 20.6% and 9.9% ($P < 0.05$). HCl-P content in *M. laosensis* and mixed stands increased by 22.2% and 12.0% ($P < 0.05$). Citrate-P content in *M. laosensis* and mixed stands increased by 20.2% and 12.0% ($P < 0.05$). In non-rhizosphere soils, $CaCl_2$-P content in *C. hystrix* and mixed stands increased by 42.6% and 28.9% ($P < 0.05$); Enzyme-P increased by 8.5% and 4.8%; HCl-P increased by 13.1% and 7.0%; and Citrate-P increased by 18.8% and 10.2% ($P < 0.05$).

As shown in [Figure 2: see original paper], following conversion, PAC in rhizosphere soils of *C. hystrix* and mixed stands was 62.2% and 60.3% higher than in Chinese fir stands ($P < 0.05$), and 78.0% and 75.9% higher than in *M. laosensis* stands ($P < 0.05$). In non-rhizosphere soils, PAC in *C. hystrix* and mixed stands was 109.3% and 111.2% higher than in Chinese fir stands ($P < 0.05$), and 124.6% and 126.7% higher than in *M. laosensis* stands ($P < 0.05$). No significant differences in PAC were observed between Chinese fir and *M. laosensis* stands in either soil type ($P > 0.05$).

2.3 Microbial Biomass and Enzyme Activity Characteristics Following Conversion

Following conversion from Chinese fir to broad-leaved forests, MBC, MBN, and MBP contents in both rhizosphere and non-rhizosphere soils were significantly higher in *C. hystrix*, *M. laosensis*, and mixed stands compared to Chinese fir stands, with greater concentrations in rhizosphere soils. In rhizosphere soils, *C. hystrix* stands showed increases of 75.5% (MBC), 62.4% (MBN), and 94.4% (MBP) ($P < 0.05$); *M. laosensis* stands increased by 144.1%, 112.2%, and 153.0% ($P < 0.05$); and mixed stands increased by 98.4%, 87.5%, and 77.8% ($P < 0.05$). In non-rhizosphere soils, *C. hystrix* stands increased by 124.6% (MBC), 106.2% (MBN), and 117.0% (MBP) ($P < 0.05$); *M. laosensis* stands increased by 266.1%, 175.4%, and 186.4% ($P < 0.05$); and mixed stands increased by 181.5%, 133.5%, and 78.7% ($P < 0.05$).

As shown in [Figure 4: see original paper], all five enzyme activities (BG, CB, NAG, LAP, ACP) were significantly higher in broad-leaved stands than in Chinese fir stands, with rhizosphere soils showing higher activities than non-rhizosphere soils. BG activity in rhizosphere and non-rhizosphere soils of *C.*

hystrix and mixed stands was significantly greater than in Chinese fir and *M. laosensis* stands ($P < 0.05$). CB, NAG, LAP, and ACP activities in rhizosphere and non-rhizosphere soils of all three broad-leaved stands were significantly higher than in Chinese fir stands ($P < 0.05$).

2.4 Key Biotic and Abiotic Factors Influencing Soil Phosphorus Fractions

RDA results revealed that in rhizosphere soils, the first and second axes explained 96.22% and 0.34% of variation in soil P fractions, respectively, with the first axis clearly separating Chinese fir stands from broad-leaved stands ([Figure 5: see original paper]a). In non-rhizosphere soils, the first and second axes explained 92.31% and 3.75% of variation, with the first axis similarly distinguishing Chinese fir from broad-leaved stands ([Figure 5: see original paper]b). Soil water content (SWC) was the most critical factor driving variation in rhizosphere soil P fractions ($F = 115$, $P = 0.002$), while microbial biomass carbon (MBC) was the key factor regulating P fractions in non-rhizosphere soils ($F = 45.8$, $P = 0.002$).

3.1 Effects of Conversion on Rhizosphere and Non-Rhizosphere Soil Phosphorus Fractions

Conversion from Chinese fir to broad-leaved forests increased all P fraction contents in both rhizosphere and non-rhizosphere soils, with consistently higher concentrations in rhizosphere soils. This likely reflects stronger root absorption and enrichment capacity for P, coupled with higher microbial activity in the rhizosphere that facilitates P mineralization. The observed pattern of HCl-P > Citrate-P > Enzyme-P > $CaCl_2$-P aligns with previous findings. $CaCl_2$-P, representing directly plant-available P through interception or diffusion, showed the lowest concentrations. In both soil types, $CaCl_2$-P was significantly higher in *C. hystrix* and mixed stands than in Chinese fir stands. Enzyme-P, a soil organic P fraction readily hydrolyzed by ACP and phytase to HPO_4^{2-} and $H_2PO_4^-$, was significantly elevated in *M. laosensis* and mixed stands. HCl-P and Citrate-P, representing stable inorganic P pools, were also significantly higher in *M. laosensis* and mixed stands. These results indicate that conversion to broad-leaved forests increases readily soluble P content, enhances P availability, and promotes accumulation of potential P pools. Possible mechanisms include: (1) increased litterfall under broad-leaved stands returns more P to soil through decomposition; (2) accumulated litter reduces P loss from exposed surfaces; and (3) different rhizosphere biochemical processes among species, including varying capacities for organic acid and enzyme secretion, create distinct P fraction profiles.

Soil P forms exist in dynamic equilibrium, with their transformations influencing P availability. In this study, increased TP, AP, and MBP following conversion may result from enhanced litterfall inputs increasing nutrient return, while higher SWC in broad-leaved stands (see) promotes microbial growth and activity, increasing MBP and affecting P accumulation. Additionally, increased P fractions elevate TP and AP, and higher SOC content in broad-leaved stands enhances P mineralization, improving P availability. These findings corroborate previous research demonstrating that establishing broad-leaved forests on Chinese fir clearcuts increases soil P availability.

3.2 Effects of Conversion on Rhizosphere and Non-Rhizosphere Soil Phosphorus Transformation

Soil P transformation involves interconversion among different P forms, influenced by vegetation type, soil physicochemical properties, microorganisms, and moisture. Acid phosphatase (ACP) plays a crucial role in P transformation, with its activity directly reflecting transformation efficiency. In this study, ACP values in *C. hystrix*, *M. laosensis*, and mixed stands were significantly higher than in Chinese fir stands, with greater activity in rhizosphere soils, indicating that conversion promotes P transformation and availability, particularly in the rhizosphere. Higher litter quantity and decomposition rates in broad-leaved stands provide abundant nutrients for microbial growth, stimulating ACP synthesis.

Conversion to broad-leaved species also increased microbial biomass (MBC, MBN, MBP) and enzyme activities (BG, CB, NAG, LAP, ACP) compared to Chinese fir plantations, primarily because higher SOC and TN contents in broad-leaved stands provide sufficient C and N sources that stimulate microbial activity, enhance enzyme activities, and promote transformation among P forms. Research on fine root characteristics indicates that Chinese fir, adapted to nutrient-poor soils, develops fewer and coarser fine roots with lower nutrient utilization efficiency compared to broad-leaved species. This suggests that conversion increases microbial biomass and activity, alters P transformation rates and nutrient competition intensity, and subsequently affects available P content.

This study identified SWC as the key environmental factor influencing rhizosphere soil P fractions, while MBC was the primary factor regulating non-rhizosphere P fractions. Previous studies have shown that increased soil moisture accelerates P mineralization and enhances phosphate diffusion from bulk to rhizosphere soil. Broad-leaved stands in this study exhibited significantly higher SWC, microbial biomass, and P fractions than Chinese fir stands, likely due to differences in litter quality and quantity affecting microbial diversity and soil moisture. Higher SWC in broad-leaved stands enhances microbial activity, promoting secretion of ACP, low-molecular-weight organic acids, and H^+. Increased Citrate-P may result from organic acids competing with phosphate ($HPO_4{}^{2-}$ and $H_2PO_4{}^-$) for anion adsorption sites on soil particles through ligand exchange, releasing adsorbed inorganic P and increasing stable inorganic P fractions. H^+ can activate insoluble mineral P, increasing HCl-P content and moderately stable inorganic P fractions, while ACP enhances mineralization of inorganic and organic P, increasing Enzyme-P content and organic P fractions.

In conclusion, conversion from Chinese fir to broad-leaved forests significantly altered rhizosphere and non-rhizosphere soil P fractions, with broad-leaved stands showing higher AP, TP, MBP, and ACP activities. SWC and MBC were identified as key drivers of P fraction variation in rhizosphere and non-rhizosphere soils, respectively. Conversion from pure Chinese fir plantations to broad-leaved forests, especially mixed stands, improved soil physicochemical properties, stimulated microbial activity, enhanced ACP activity, promoted mineralization of organic P and dissolution of inorganic P, and favored soil P storage and transformation. These changes increase plant nutrient supply, indirectly affect subtropical forest productivity, and contribute to sustainable plantation ecosystem management.

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