Leaf-Root-Soil Nitrogen and Phosphorus Stoichiometric Characteristics of Subtropical Chinese Fir Forests at Different Stand Ages: Postprint

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Abstract

Taking the needles, fine roots, and soil (0-15, 15-30, 30-45 cm) of Chinese fir (Cunninghamia lanceolata) plantations aged 5, 10, 15, 20, and 25 years in Huitong, Hunan, a subtropical region, as research objects, and based on measurements of total N and total P contents in needles, fine roots, and soil, this study explored changes in N and P stoichiometric characteristics of the needle-root-soil system throughout the entire life cycle of Chinese fir plantations to provide fundamental data for their management. The results showed that: (1) Stand age had an extremely significant effect on soil N and P contents and N:P ($P < 0.01$). Soil layer had a significant effect on soil N content ($P < 0.01$). Soil N and P contents in each layer exhibited a trend of first decreasing then increasing with stand age, with significant changes ($P < 0.05$). The maximum values of soil N and P contents appeared in the mature forest and young forest stages, respectively, while the minimum values appeared in the middle-aged forest stage. Soil N:P showed an increasing trend with stand age, but the change was not significant. (2) Both stand age and organ had extremely significant effects on plant N and P contents and N:P ($P < 0.01$). The N and P contents in needles and fine roots showed a "V-shaped" trend with stand age, with significant changes ($P < 0.05$). The maximum values of N and P contents in both needles and fine roots appeared in the young forest and mature forest stages, while the minimum values appeared in the middle-aged forest stage. The N:P of Chinese fir needles did not change significantly with stand age, whereas the N:P of fine roots increased significantly with stand age ($P < 0.05$). The N:P ranges of Chinese fir needles and fine roots were 11.79-14.86 and 9.00-22.89, respectively. (3) Across the five stand ages, N and P contents in needles, fine roots, and soil all showed the pattern of needle > fine root > soil, with significant differences ($P < 0.05$). The N and P contents and N:P in needles and fine roots were significantly positively correlated ($P < 0.05$). The 0-15 cm soil N was not significantly correlated with needle and fine root N, while the 15-30 cm and 30-45 cm soil N showed significant correlations with needle and fine root N at ages 5 and 10 years (P < 0.05). Across the five stand ages, significant correlations existed among the P contents and N:P of Chinese fir needles, fine roots, and soil. These results indicate that during the growth process of Chinese fir, nutrients in needles, fine roots, and soil continuously change, and the N and P stoichiometric characteristics among needles, fine roots, and soil show certain correlations.

Full Text

Variation in the N and P Stoichiometry of Leaf–Root–Soil Systems During Stand Development in a *Cunninghamia lanceolata* Plantation in Subtropical China

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Abstract

We measured total nitrogen (N) and phosphorus (P) concentrations in leaf, fine root, and soil (0–15, 15–30, 30–45 cm) samples from 5-, 10-, 15-, 20-, and 25-year-old *Cunninghamia lanceolata* plantations in Huitong, Hunan Province. The objective was to determine variation in N and P stoichiometry of leaf–root–soil systems throughout the entire life cycle of *C. lanceolata* plantations and provide baseline data to guide plantation management. The results showed: (1) Stand age had a significant effect on soil N and P concentrations and N:P ratios ($P < 0.01$), while soil depth only significantly affected soil N concentration ($P < 0.01$). Soil N concentration decreased with increasing soil depth, whereas soil P concentration and N:P ratio showed no significant difference across depths. In each soil layer, N and P concentrations initially decreased then increased significantly with stand age ($P < 0.05$). Soil N and P concentrations peaked in 25-year and 5-year plantations respectively, and reached minima in 10-year and 15-year plantations. The soil N:P ratio increased with stand age, though not significantly. (2) Stand age and organ type had significant effects on plant N and P concentrations and N:P ratios ($P < 0.01$). Leaf and fine root N and P concentrations showed a significant "V-shaped" pattern with stand age ($P < 0.05$), except for fine root P, which decreased slightly in 20-year plantations. Leaf and fine root N and P concentrations were highest in 5-year and 25-year plantations and lowest in 15-year plantations. Leaf N:P ratio did not change significantly with stand age (range: 11.79–14.86). For all stand ages, leaf N:P ratios were below 14, except in 20-year plantations, indicating N was the primary limiting factor for *C. lanceolata* growth. Fine root N:P ratio increased significantly with stand age ($P < 0.05$; range: 9.00-22.89). (3) Throughout the plantation life cycle, N and P concentrations differed significantly among leaf, fine root, and soil in the order: leaf > fine root > soil ($P < 0.05$). Significant correlations existed between leaf and fine root N and P stoichiometry ($P < 0.05$), except for leaf and fine root N in 25-year plantations. No significant correlations were found between 0–15 cm soil and leaf/fine root N concentrations. However, significant correlations occurred between 15–30 cm and 30–45 cm soil and leaf/fine root N and P concentrations in 5- and 10-year plantations ($P < 0.05$), but not in 15-, 20-, and 25-year plantations. Throughout the life cycle, significant correlations existed between leaf/fine root and soil P concentrations and N:P ratios. We conclude that forest development alters leaf, fine root, and soil nutrients, reflecting strong linkages in N and P stoichiometry among these components.

Keywords: leaf-root-soil; different stand ages; ecological stoichiometry; nitrogen; phosphorus; *Cunninghamia lanceolata*

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1. Study Area Overview

The study site was located at the Chinese Fir Base of Guangping Town Ecological Station in Huitong County, Hunan Province, the central production area of Chinese fir in China. Geographically situated at 109°45 E, 26°50 N, with an elevation of 250–500 m, the region has a typical subtropical humid climate. The topography consists of low mountains and hills. The annual average temperature is 16.8°C, with annual precipitation of 1,422 mm and 1,677.1 hours of sunshine. The frost-free period lasts 270–300 days, and annual relative humidity is 83%. Soils are mountain yellow soils developed from Sinian Banxi series gray-green slate, with pH between 4.31–4.86 and texture ranging from medium loam to medium clay loam—conditions favorable for Chinese fir growth.

Vegetation in the sample plots is dominated by *Cunninghamia lanceolata*, with minor species including *Quercus fabri*, *Vernicia fordii*, and *Alnus cremastogyne*. Understory shrubs include *Ilex chinensis*, *Maesa japonica*, and *Smilax china*, while herbaceous species consist mainly of *Dicranopteris dichotoma*, *Woodwardia japonica*, and *Cyclosorus parasiticus*. The laboratory research base is the National Engineering Laboratory for Applied Technology of Forestry and Ecology in South China. Basic plot information and soil physicochemical properties are detailed in Tables 1 and 2.

Table 1 General information of sample plots
Table 2 Physical and chemical properties of soil in different-aged *Cunninghamia lanceolata* plantations

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2. Methods

2.1 Sample Plot Setup Using a space-for-time substitution approach, we established 20 m × 20 m sample plots in 5-, 10-, 15-, 20-, and 25-year-old *Cunninghamia lanceolata* plantations. All stands were planted after clear-cutting and had received no fertilization in the previous five years. Plots were established under similar soil types and site conditions. Three replicate plots were set up for each stand age, with approximately 10 m spacing between plots of the same age.

2.2 Soil and Plant Sample Collection Samples were collected seasonally. In each plot, one healthy *C. lanceolata* tree was randomly selected. Soil samples were collected at 0–15, 15–30, and 30–45 cm depths (the 45 cm depth had many stones) after removing surface litter. Soil fresh weight was measured, and temperature and moisture were determined using a ProCheck handheld multi-parameter meter. Four soil samples were taken per plot and combined into one composite sample.

Leaf samples were collected simultaneously from the middle canopy in east, west, south, and north directions, mixing all leaves from one branch into a single sample. Four leaf samples were collected per plot. Fine roots ($<$ 2 mm diameter) were collected from the soil profile, with 100 g of fine roots sampled per plot to obtain four fine root samples.

2.3 Sample Processing and Measurement Soil samples were air-dried in the laboratory, weighed to determine moisture content and bulk density, then sieved (0.25 mm) after removing roots and stones for total N and P analysis. Leaves and fine roots were oven-dried at 65°C to constant weight, then ground and sieved (0.25 mm). Total N was determined by the Kjeldahl method, and total P by molybdenum-antimony colorimetry. Soil pH was measured with a digital pH meter (PHS-25A). Diameter at breast height (DBH) was measured with a diameter tape.

2.4 Data Analysis Data were preprocessed using Excel 2010. Differences among stand ages were tested using One-Way ANOVA in SPSS 19.0. Univariate multi-factor ANOVA was used to analyze influencing factors. Pearson correlation analysis was performed, and figures were created using SigmaPlot 12.5.

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3. Results and Analysis

3.1 Soil Stoichiometric Characteristics Across Stand Ages Stand age had extremely significant effects on soil N and P concentrations and N:P ratios ($P < 0.01$), while soil depth only significantly affected soil N concentration ($P < 0.01$). Soil N concentration decreased with depth, whereas soil P concentration and N:P ratio showed no significant depth-related differences. The interaction between stand age and soil depth had no significant effect on soil N, P, or N:P ratios.

In each soil layer, N and P concentrations initially decreased then increased significantly with stand age ($P < 0.05$). Soil N and P concentrations ranged from 1.50–1.76, 1.22–1.59, and 0.94–1.39 g/kg in the 0–15, 15–30, and 30–45 cm layers, respectively. The 0–15 cm layer showed significant stand age effects, with concentrations decreasing then increasing, peaking at 25 years and reaching minima at 10–15 years. The 15–30 cm and 30–45 cm layers showed identical trends of significant decrease followed by significant increase ($P < 0.05$). Soil N:P ratios ranged from 4.96–6.71, 3.46–6.64, and 4.00–5.91 across layers, increasing with stand age but not significantly ($P > 0.05$).

Table 3 Effects of stand age, soil depth, and their interactions on soil N, P, and N:P ratios in *Cunninghamia lanceolata* plantations

Figure 1 [Figure 1: see original paper] Changes in soil N, P, and N:P ratios with soil depth and stand age in *C. lanceolata* plantations (mean ± SE, n = 16). Different uppercase letters indicate significant differences among stand ages ($P < 0.05$); different lowercase letters indicate significant differences among soil layers within the same stand age ($P < 0.05$).

3.2 Leaf and Fine Root Stoichiometric Characteristics Across Stand Ages Organ type had significant effects on plant N and P concentrations ($P < 0.05$). Stand age significantly affected N:P ratios ($P < 0.05$), while the interaction between stand age and organ significantly influenced N and P concentrations ($P < 0.05$).

Leaf N and P concentrations were significantly higher than fine root concentrations across all stand ages. Leaf and fine root N and P concentrations showed consistent "V-shaped" patterns with stand age, decreasing to minima at 15 years then increasing significantly. Leaf N and P concentrations peaked at 5 years (14.45 ± 0.61 and 1.22 g/kg) and reached minima at 15 years (9.30 ± 0.70 and 0.57 ± 0.04 g/kg). Fine root N and P concentrations ranged from 6.46 ± 0.31 to 12.42 ± 0.36 g/kg and 0.32–0.75 g/kg, respectively.

Leaf N:P ratio showed no significant change with stand age (range: 11.79–14.86), while fine root N:P ratio increased significantly ($P < 0.05$; range: 9.00–22.89).

Table 4 Effects of stand age, organ, and their interactions on plant N, P, and N:P ratios in *Cunninghamia lanceolata* plantations

Figure 2 [Figure 2: see original paper] Changes in leaf and fine root N, P, and N:P ratios with stand age in *C. lanceolata* plantations (mean ± SE, n = 16). Different uppercase letters indicate significant differences among stand ages ($P < 0.05$); different lowercase letters indicate significant differences between leaf and fine root within the same stand age ($P < 0.05$).

3.3 Correlations Among Leaf, Fine Root, and Soil Stoichiometry Significant positive correlations existed between leaf and fine root N and P concentrations across stand ages ($P < 0.05$), except for N in 25-year plantations. Leaf N:P ratio was significantly negatively correlated with fine root N:P ratio in 5- and 10-year plantations (coefficients: -0.617 and -0.535, $P < 0.05$).

Significant positive correlations occurred between leaf/fine root N and P concentrations and 15–30 cm and 30–45 cm soil N and P concentrations in 5- and 10-year plantations ($P < 0.05$), but not in older stands. Leaf and fine root P concentrations and N:P ratios were significantly correlated with soil P concentrations and N:P ratios across all stand ages.

Table 5 Correlation analysis of total N, total P, and N:P ratios among leaf, fine root, and soil across five stand ages

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4. Discussion

4.1 Soil N and P Stoichiometric Characteristics Mean soil total N and P concentrations across all stand ages were (1.40 ± 0.03) and (0.33 ± 0.01) g/kg, respectively—higher than the Chinese soil averages of 1.06 and 0.65 g/kg, and also higher than values from the Qianyanzhou subtropical plantation (0.86 and 0.11 g/kg). This indicates that Huitong, as the central production area for Chinese fir, has unique ecological conditions favorable for soil fertility conservation. However, both study area and Qianyanzhou soils show lower N:P ratios than the Chinese average, consistent with P deficiency in low-latitude regions.

Stand age significantly affected soil N and P concentrations, which initially decreased then increased with stand age, with minima in middle-aged stands (10–15 years). This pattern aligns with studies on evergreen broadleaf forests in Yunnan and Chinese fir plantations in Hunan. The decline during middle age may result from high nutrient demand during rapid growth stages (timber phase) and low microbial activity reducing litter nutrient return. The subsequent increase in mature stands (20–25 years) reflects reduced nutrient demand and increased litter return, indicating improved soil fertility.

Soil depth significantly affected N but not P or N:P ratios. Surface soil (0–15 cm) had significantly higher N than deeper layers, likely due to litter decomposition and atmospheric N deposition causing vertical differentiation. Soil P is primarily influenced by parent material weathering—a slow process resulting in minimal depth differences.

4.2 Plant N and P Stoichiometric Characteristics Mean leaf total N and P concentrations were (12.42 ± 0.36) and (1.07 ± 0.04) g/kg, respectively—higher than values from Qianyanzhou but lower than Chinese terrestrial plant averages. Fine root N and P concentrations were (10.88 ± 0.75) and (0.75

± 0.04) g/kg, also lower than Chinese averages but higher than Qianyanzhou values. These patterns reflect adaptation to low-latitude conditions.

Organ type was the primary factor influencing N and P concentrations, reflecting functional differentiation in nutrient absorption and utilization. Leaf N and P concentrations were consistently higher than fine root concentrations, and the significant positive correlations between leaf and fine root nutrients indicate that leaf nutrient status derives from fine root uptake.

Stand age significantly affected plant N and P concentrations, which showed "V-shaped" patterns with minima at 15 years. This reflects changing nutrient demand during stand development. The initial decline corresponds to rapid growth phases with high nutrient requirements, while the subsequent increase suggests nutrient accumulation as growth slows.

4.3 Correlations Among Leaf, Fine Root, and Soil Stoichiometry Significant correlations between leaf and fine root N:P ratios in early growth stages (5–10 years) demonstrate tight coupling in nutrient allocation. The lack of correlation between 0–15 cm soil and plant N concentrations suggests that surface soil nutrients may not directly reflect plant nutrient status, possibly due to preferential fine root distribution in deeper layers.

In young plantations (5–10 years), significant correlations between deeper soil layers (15–45 cm) and plant nutrients indicate that trees rely on these layers during early growth. The disappearance of these correlations in older stands suggests that internal nutrient cycling becomes more important as plantations mature.

The consistent significant correlations between plant and soil P concentrations and N:P ratios across all stand ages highlight the critical role of soil P availability in regulating plant nutrient balance throughout stand development.

4.4 Nutrient Limitation Indicators The leaf N:P ratio, an indicator of nutrient limitation, averaged 13.06 ± 0.64 across all stand ages—below the critical value of 14, indicating N limitation. This is consistent with previous studies in Huitong. The fine root N:P ratio (15.97 ± 1.25) was higher than the Chinese average, suggesting efficient P uptake and utilization under N-limited conditions.

The "growth rate hypothesis" posits that rapidly growing organisms allocate more P to ribosomes for protein synthesis, resulting in low N:P ratios. The increasing fine root N:P ratio with stand age in this study suggests decreasing growth rates as plantations mature.

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5. Conclusion

Stand age significantly affects N and P stoichiometry in leaf, fine root, and soil components of *Cunninghamia lanceolata* plantations. Soil N and P concentrations show "V-shaped" patterns with minima in middle-aged stands, while N:P ratios increase with stand age. Plant nutrients are consistently higher than soil nutrients, with leaves > fine roots > soil. Strong correlations exist between leaf and fine root stoichiometry, and between plant and soil P status. These patterns reflect the dynamic nutrient cycling and allocation strategies throughout the plantation life cycle, providing a scientific basis for managing Chinese fir plantations across different developmental stages.

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Note: Figure translations are in progress. See original paper for figures.