

Increasing stand age increases N deficiency but alleviates relative P limitations in *Castanopsis hystrix* plantations in southern China

Xu LI¹, Ting Wu¹, Zhiyang Lie¹, Luis Carlos Ramos Aguila¹, Yue Liu¹, Yan Cheng¹, Fen Jiang¹, Xujun Liu¹, and ¹

¹South China Botanical Garden

June 28, 2023

Abstract

Aims Forest productivity may decrease with increasing stand age, which may be related to a shift in the nitrogen (N) and phosphorus (P) nutrient balance. However, it is unclear how the plantation nutrient balance changes with stand age and how it affects plantation productivity. **Methods** We experimented with increasing stand ages (6-, 10-, 15-, 25-, 30-, and 34-year-old stands) in *Castanopsis hystrix* plantations in southern China. Plant growth and the carbon (C), N, and P dynamics were assessed at the ecosystem level (leaf–litter–soil–microorganism). **Results** Increasing stand ages reduced plantation productivity and leaf N:P ratios (lower leaf N concentrations and stable leaf P concentrations). The reduced productivity may be related to increased N consumption rather than possible P limitations. Increasing stand ages increased N consumption mainly through decreasing soil NO₃⁻-N concentrations and microbial biomass without altering plant N resorption, although enhanced N-acetylglucosaminidase, cellobiohydrolase, and polyphenol oxidase activity might accelerate N mineralization. Increased soil total P concentrations and microbial biomass P rather than plant P resorption would support a larger P supply for plants with increasing stand ages. **Conclusions** These results suggest that increasing stand ages can contribute to N consumption rather than elevate possible P limitations. Therefore, N fertilizer management should receive attention to maintain the productivity of *C. hystrix* plantations with increasing stand age.

Increasing stand age increases N deficiency but alleviates relative P limitations in *Castanopsis hystrix* plantations in southern China

Xu Li^{a,b,c,1}, Ting Wu^{a,b,1}, Zhiyang Lie^{a,b}, Luis Carlos Ramos Aguila^{a,b}, Yue Liu^{a,b,c}, Yan Cheng^{a,b,c}, Fen Jiang^{a,b,c}, Xujun Liu^{a,b,c}, Juxiu Liu^{a,b*}

^aKey Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, 723 Xingke Road, Guangzhou 510650, China

^bGuangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, 723 Xingke Road, Tianhe District, Guangzhou, 510650, China

^cUniversity of Chinese Academy of Sciences, Beijing 100049, China

¹ Xu Li and Ting Wu contributed equally to this work.

* Corresponding author: Juxiu Liu

Address: No.723, Xingke Road, Tianhe District, Guangzhou, China

E-mail address: ljxiu@scbg.ac.cn

Abstract

Aims Forest productivity may decrease with increasing stand age, which may be related to a shift in the nitrogen (N) and phosphorus (P) nutrient balance. However, it is unclear how the plantation nutrient balance changes with stand age and how it affects plantation productivity.

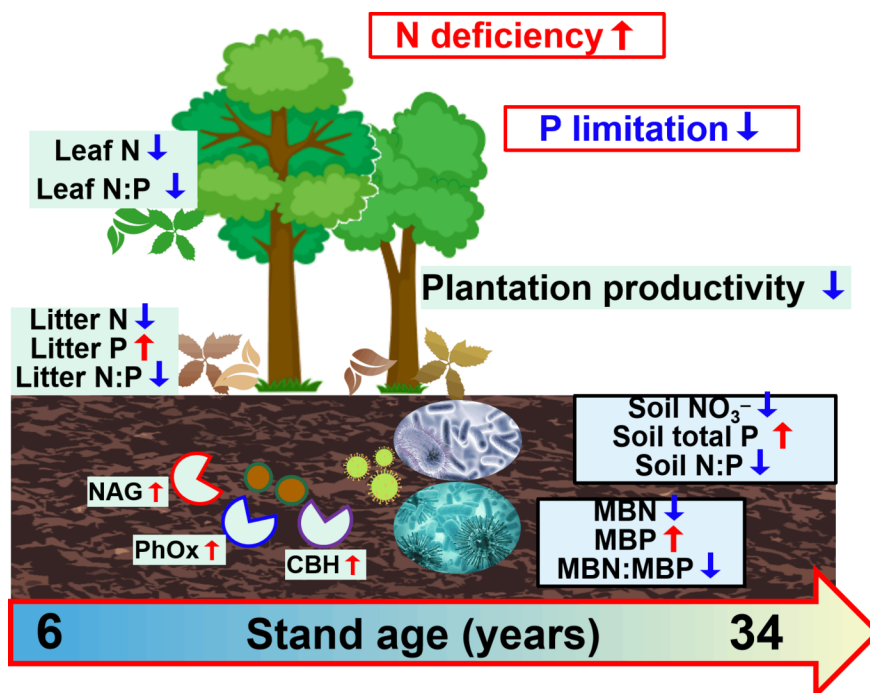
Methods We experimented with increasing stand ages (6-, 10-, 15-, 25-, 30-, and 34-year-old stands) in *Castanopsis hystrix* plantations in southern China. Plant growth and the carbon (C), N, and P dynamics were assessed at the ecosystem level (leaf–litter–soil–microorganism).

Results Increasing stand ages reduced plantation productivity and leaf N:P ratios (lower leaf N concentrations and stable leaf P concentrations). The reduced productivity may be related to increased N consumption rather than possible P limitations. Increasing stand ages increased N consumption mainly through decreasing soil NO_3^- -N concentrations and microbial biomass without altering plant N resorption, although enhanced N-acetylglucosaminidase, cellobiohydrolase, and polyphenol oxidase activity might accelerate N mineralization. Increased soil total P concentrations and microbial biomass P rather than plant P resorption would support a larger P supply for plants with increasing stand ages.

Conclusions These results suggest that increasing stand ages can contribute to N consumption rather than elevate possible P limitations. Therefore, N fertilizer management should receive attention to maintain the productivity of *C. hystrix* plantations with increasing stand age.

Keywords : Nutrient dynamics; Nitrogen; Phosphorus; Leaf; Tropical forests

Graphical abstract



1. Introduction

Forests are the largest terrestrial carbon (C) sinks due to their high capacity to store large amounts of C in plants and soils (Hua et al., 2022). Human overuse of land has reduced forest area and C sinks in forests over the past 30 years (FAO, 2020; Yuan and Chen, 2012; Hua et al., 2022). Therefore, afforestation and the protection of existing forests are important measures to improve the C sequestration capacity of terrestrial ecosystems, which can mitigate climate change (Bonner et al., 2013; Chen et al., 2019). China has made a

significant contribution to global greening, with intensive afforestation projects (e.g., plantations) accounting for 42% of this contribution (Chen et al., 2019). The nutrient status in subtropical plantations changes with increasing stand age, and understanding the association between nutrient status and stand age would be helpful for devising strategies to sustain and improve ecosystem stability and C sequestration capacity (Feng et al., 2017; Yang et al., 2021).

Subtropical forests in China possess a great diversity of plants and provide high net C productivity (Yu et al., 2014). Nutrient limitation is defined as an increase in plant productivity with an increase in nutrient availability (Elser et al., 2007). Nitrogen (N) and/or phosphorus (P) often limit plant productivity in most terrestrial ecosystems (Vitousek and Howarth, 1991; Elser et al., 2007; Vitousek et al., 2010). Leaf N:P ratios can indicate the nutrient limitation of plants (Gusewell, 2004; Bui and Henderson, 2013; Liu et al., 2013a). In tropical and subtropical areas, young plantations are generally vulnerable to N limitation, while older plantations are limited by P (Fan et al., 2015; Wang and Zheng, 2021; Yang et al., 2021). Hence, P could become increasingly limiting to plant growth with increasing stand age. Yang et al. (2021) have reported that N limitation decreases while P limitation increases with increasing stand age in subtropical southeast China. Therefore, it is important to focus on the change in the nutrient balance with forest age in the subtropics.

Nutrient limitation in plantation forests is related to soil nutrient availability. Changeable N and P concentrations in plants can result in a change in the N:P ratio, which exerts a significant impact on plant productivity and the process of litter decomposition (Feng et al., 2019b). The C:N ratios and C:P ratios in litter and soil have been recognized as quality indicators of organic matter and its decomposition rate (Paul et al., 2002; Zhang et al., 2013; Liu et al., 2017; Yang et al., 2021). Soil N and P concentrations change with the stand age of forests due to factors including the changeable litter biomass, enzyme activities, and microbial community (Deng et al., 2013; Lucas-Borja et al., 2016; Moreno-Mateos et al., 2017; Feng et al., 2019a; Feng et al., 2019b). Increased stand ages result in higher litter biomass, which elevates soil N concentrations (Moreno-Mateos et al., 2017; Feng et al., 2019b). Higher microbial biomass, abundance, and diversity caused by increasing stand ages enhance the soil available N concentrations (Deng et al., 2013; Moreno-Mateos et al., 2017). In addition, higher enzyme activities, including polyphenol oxidase (PhOx) and N-acetylglucosaminidase (NAG) activities, contribute to increasing the available N concentrations through accelerating litter decomposition (Moreno-Mateos et al., 2017; Feng et al., 2019b; Dong et al., 2021). However, lower soil total P and available P concentrations are expected to be found in older forests than in younger forests (Frizano et al., 2002; Feng et al., 2017; Chen et al., 2018; Yang et al., 2021), which is driven by higher P accumulation in plants (Kitayama et al., 2000; Vitousek et al., 2010; See et al., 2015). Lower soil available P concentrations under increasing stand ages may be due to reduced acid phosphomonoesterase (AP) activity (Wang et al., 2019; Yang et al., 2020). Microbial biomass N (MBN) and P (MBP) can also reflect the status of soil N and P, and MBN and MBP can be converted into soil available N and P for plants (Lucas-Borja et al., 2016). Some studies have shown that soil MBN and MBP increase with stand ages due to increasing litter biomass and enzyme activity (Feng et al., 2019a; Feng et al., 2019b; Wang et al., 2019). However, it is not clear whether soil available P decreases and available N increases with increasing plantation age in N-rich and P-deficient subtropical regions, and thus increasing the P-limitation of plantation forests.

Castanopsis hystrix plantation occupies more than 50, 000 ha in southern China and provides high C storage and economic benefits (He et al., 2013; Zhou et al., 2013; Xu et al., 2020). However, the vegetation dynamics and the relationship between soil development and vegetation in *C. hystrix* plantations have rarely been studied. Previous studies have investigated the implications of increasing stand ages on the alternations in nutrient concentrations and stoichiometry in single components of many plantations, but have not explained the ecosystem-level (leaf-litter-soil-microorganism) nutrient limitation mechanisms (Feng et al., 2019a; Feng et al., 2019b; Wang and Zheng, 2021; Yang et al., 2021). This study examined the impacts of increasing stand ages (6-, 10-, 15-, 20-, 30-, and 34-year-old stands) on the nutrient concentrations and stoichiometry in the leaves, litter, soil, microorganisms, and soil enzyme activities in a *C. hystrix* plantation in southern China. The main objectives of this study were to (a) investigate the impacts of increasing stand ages on

the nutrient concentrations and stoichiometry in the leaves, litter, soil, and microorganisms of the *C. hystrix* plantation; (b) determine the mechanisms regulating the nutrient concentrations in the soil, microorganisms, and plants. High N deposition with 30–50 kg N ha⁻¹ yr⁻¹ has been reported in some studies (Fang et al., 2008; Hietz, 2011), and P deficiency is often found in forest ecosystems in southern China. Thus, in the present study, it was hypothesized that increasing stand ages in the *C. hystrix* plantation would (a) decrease aboveground productivity and (b) increase leaf N:P ratios due to stable leaf N concentrations and decreased P concentrations, and that (c) the reduced productivity might be related to increased P consumption.

2. Materials and methods

2.1 Study area

The study was conducted at the Longyandong Forest Farm (113°21' E–113°27' E and 23°10' N–23°18' N) in southern China. The study area has a tropical monsoon climate that is characteristic of the area. Throughout the year, the average temperature is 21 °C and the relative humidity is 80%. There are approximately 1,900 mm of precipitation per year in the area, during the wet season (April–September), the region receives approximately 80% of rainfall; while, in the dry season (October–March), it receives approximately 20% (Liu et al., 2013). The region has experienced considerable N deposition (34 kg N ha⁻¹ yr⁻¹) due to rapid urbanization since 1978 (Huang et al., 2015). The soil type is lateritic red soil and is mainly composed of granite and sand shale.

C. hystrix plantations were planted in 1986, 1990, 1995, 2005, 2010, and 2014 after clear cutting. All plantations created in different years were subjected to the same artificial cultivation measures, including fertilization and the removal of understory vegetation, in the third and fifth years, followed by the cessation of artificial disturbance. All lands had similar geological and land use history before planting; all lands were planted with *Acacia mangium* using the same methods beginning in the 1970s, before which all lands were covered with evergreen broad-leaved forest, dominated by *C. hystrix*, *Castanopsis chinensis*, *Syzygium rehderianum*, and *Schima superba* (Zhou et al., 2013). The main shrub species were *Ficus hirta*, *Psychotria asiatica*, and *Melicope pteleifolia*, and the main herb species were *Lophatherum gracile*, *Woodwardia japonica*, and *Blechnum orientale*.

2.2 Plot design

After carefully selecting and replicating plots, a chronosequence approach was used to survey forest stands (Walker et al., 2010). In August 2020, a sample survey was conducted in six stands of different ages (6, 10, 15, 25, 30, and 34 years old). Three plots (20 m x 20 m) were randomly set up in each stand (a total of 18 plots; three plots x six stand ages). A distance of at least 20 m was maintained between each plot and the forest edge, and the slope positions and aspects of all selected plots were similar. Plots between different stand ages were spaced at least 1 km apart to minimize spatial autocorrelation. The data of all stand ages were obtained from the records of the Longyandong Forest Farm (Table 1).

2.3 Field measurements

In August 2020, mature leaves and litter (1 m x 1 m on the ground) were collected at each stand age of the *C. hystrix* plantation (three replicates x six stand ages = 18 samples). All live trees were measured simultaneously to determine the diameter at breast height (DBH, cm; [?] 5 cm) and tree height (m). The species richness and stand density (tree ha⁻¹) were calculated by summing the stand basal area (m² ha⁻¹) for each species at the plot level (Feng et al., 2017; Yang et al., 2021).

Seven subsamples were collected from each of the three soil layers (0–10, 10–20, and 20–40 cm) using a 4-cm diameter auger, and the three subsamples from each soil layer were combined into a single composite

sample. The soil samples were quickly refrigerated using ice packs and handheld storage boxes so they could be tested in the laboratory within 72 hours.

2.4 Aboveground productivity of *C. hystrix* plantations

The tree and understory biomass were measured in each plot of the *C. hystrix* plantation in August 2020. The tree biomass was estimated using allometric equations that correlated biomass with the tree height and DBH (Zhou et al., 2018). All understory vegetation (shrubs and herbs) was harvested from three sub-quadrats (2 m x 2 m) randomly located in each plot. Litter samples were collected from three sub-quadrats (1 m x 1 m) located within each plot. Then, all plant samples were dried at 65degC to obtain a constant weight for determining the biomass. The annual aboveground productivity of the *C. hystrix* plantation was calculated as the aboveground (tree + understory + litter) biomass/stand age.

2.5 Laboratory assessments

A minimum of 72 hours were required for the oven drying of the leaf and litter samples. The Walkley–Black wet digestion method as described by Nelson and Sommers (1982) was used to determine the concentrations of C in leaf and litter samples (g kg^{-1}). The Kjeldahl method (Bremner and Mulvaney, 1982) was used to determine the N concentrations (g kg^{-1}) in leaf and litter samples. The leaf and litter P concentrations (g kg^{-1}) were measured using a photometer after digesting the samples with $\text{H}_2\text{SO}_4\text{--H}_2\text{O}_2$.

An ultraviolet spectrophotometer was used to determine the concentrations (mg kg^{-1}) of NO_3^- -N and NH_4^+ -N in soil that had been extracted with KCl solution at 1 M. A solution containing 0.03 M NH_4F and 0.025 M HCl was used to extract the soil available P concentration (mg kg^{-1}) (the ratio between soil and extractant was 1:7). Then, an ultraviolet spectrophotometer was used to measure the results. Dichromate oxidation and titration with ferrous ammonium sulfate as described by Nelson and Sommers (1982) were used to determine the soil organic C (SOC, g kg^{-1}).

The activities of soil enzymes involved in the cycling of C, N, and P were measured, as well as four hydrolytic enzymes, namely cellobiohydrolase (CBH), β -glucosidase (BG), AP, and NAG, and two oxidases, namely peroxidase and PhOx. The methodology described by Lie et al. (2019) was used to measure the BG, CBH, NAG, and AP activities. Hydrolytic enzymes were measured colorimetrically using a Multiskan EX (Thermo Scientific, Waltham, MA, USA) at 405 nm, and oxidase enzymes were measured at 450 nm (Tabatabai, 1994). To calculate the specific enzyme activities, this study followed the methodology described by Trasar-Cepeda et al. (2007) and divided the enzyme activities by the soil microbial biomass carbon (MBC).

The fumigation–extraction method was used to analyze the soil MBC, MBN, and MBP (Vance et al., 1987). The organic C and N of moist soil were extracted using a solution containing 0.5 M K_2SO_4 , while P was extracted with 0.03 M NH_4F and 0.025 M HCl. Following fumigation with chloroform for 24 hours at 25°C, the same extraction methods were conducted. A Total Organic Carbon Analyzer (TOC-VCSH, Shimadzu, Japan) was used to determine the extracted C and N in K_2SO_4 , while the extracted P was determined using inductively coupled plasma optical emission spectroscopy. Based on the differences between the fumigated and unfumigated subsamples multiplied by their conversion factors, the microbial biomass values were calculated (Jenkinson et al., 2004).

2.6 Statistical analysis

To understand the mechanisms associated with the indirect and direct effects of altered leaf, litter, and soil nutrients triggered by stand ages, linear regression was used to examine the associations between stand ages and leaf, litter, soil characteristics, and microbial parameters. The relationships between variables (leaf, litter, and soil C:N:P concentrations and ratios) were represented by Pearson correlation coefficients. To determine the effects of stand ages, soil depth, and their interactions on soil characteristics and microbial

parameters, two-way analyses of variance (ANOVAs) were used. All analyses were performed in R 4.0.4. and SPSS 20.0. The level of statistical significance was set at 0.05.

3. Results

3.1 Effects of increasing stand ages on the nutrient concentrations and ratios in leaves and aboveground productivity

Increasing stand ages did not affect leaf P concentrations but significantly reduced leaf N concentrations and the N:P ratio (Figure 1b and f, $P < 0.05$). Additionally, increased leaf C concentrations and C:N ratios were found in *C. hystrix* plantations with increasing stand ages (Figure 1a and d, $P < 0.05$).

Increasing stand ages significantly reduced the aboveground productivity (Figure 2a, $P < 0.05$). The aboveground productivity was positively correlated with leaf N:P ratios (Figure 2b, $P < 0.01$).

3.2 Effects of increasing stand ages on the nutrient concentrations and ratios in litter

The litter C and P concentrations and C:N ratio were enhanced with increasing stand ages (Figure 3a, c and d, $P < 0.05$); however, the litter N concentrations and N:P ratio were decreased with increasing stand ages (Figure 3b and f, $P < 0.05$).

3.3 Effects of increasing stand ages on soil properties

The soil organic C, total P concentrations, and C:N ratio in the three soil layers were significantly enhanced with increasing stand ages (Figure 4a, c, and d, $P < 0.05$). However, the soil C:P and N:P ratios decreased with increasing stand ages (Figure 4e and f, $P < 0.05$).

Increasing stand ages decreased the soil NO_3^- -N concentrations in three soil layers (Figure 5a, $P < 0.05$) but had no significant change in the soil NH_4^+ -N concentrations (Figure 5b). The soil available N concentrations in the 20–30 cm soil layer and the available P concentrations in the 0–10 cm soil layer were reduced with increasing stand ages (Figure 5c and d, $P < 0.05$).

The MBC and MBC:MBP ratios in the top (0–10 cm) and middle (10–20 cm) layers were reduced with increasing stand ages (Figure 6a and e, $P < 0.05$). The MBN and MBN:MBP in the top layer (0–10 cm) and the MBC:MBN in the middle layer (10–20 cm) displayed a declining trend with higher stand ages (Figure 6b, d, and f, $P < 0.05$). In contrast, increasing stand ages led to higher MBP in the top layer (0–10 cm) (Figure 6c, $P < 0.05$).

The AP activity decreased with increasing stand ages in the deep soil layer (20–30 cm) (Figure 7a, $P < 0.05$). The PhOx and NAG activities in the top layer (0–10 cm) and the CBH activity in the middle layer (10–20 cm) were enhanced with increasing stand ages (Figure 7b, c, and d, $P < 0.05$). The BG activity initially increased and then decreased as the stand age increased in all soil layers (Figure 7e, $P < 0.05$). In contrast, the peroxidase activity initially decreased and then increased as the stand age increased in the top and middle layers (Figure 7f, $P < 0.05$).

4. Discussion

4.1 Nutrient limitations and productivity in *C. hystrix* plantations

Consistent with the hypothesis, increasing stand ages reduced plantation productivity, which might have been caused by increased N consumption rather than possible P limitations. As forest stands age, P increasingly accumulates in aboveground biomass or in the soil in recalcitrant organic and inorganic forms, which further decreases the biological availability of P (Vitousek & Farrington, 1997; Lambers et al., 2008; Wardle et al., 2008). During forest succession, species use different strategies to acquire P, including resorption from foliage or/and absorption from the soil via rhizosphere processes.

The alternations in nutrient concentrations and stoichiometry caused by increasing stand ages alter forest properties and functions, such as lowering the capacity and rate of C fixation and productivity in plantations (Fan et al., 2015; Ma et al., 2017). Therefore, knowing the effects of increasing stand ages on nutrient

status would be helpful to take measures to maintain ecosystem function in plantations, such as adding fertilizer (Waterworth et al., 2007; Markewitz et al., 2012; Zhang et al., 2022). Most studies have shown that P limitation is strengthened by increasing stand ages in subtropical plantations due to accumulation in plants (Fan et al., 2015; Yang et al., 2021). Additionally, lower N:P ratios have been found in older *C. hystrix* plantations (leaf N:P < 20) than younger ones (leaf N:P > 20), suggesting that increasing stand ages increase N limitations but reduce P limitations in *C. hystrix* plantations (Gusewell, 2004; Bui and Henderson, 2013; Liu et al., 2013; Yang et al., 2021). Overall, increasing stand ages intensify N limitations but not P limitations in *C. hystrix* plantations, and reduced leaf N concentrations may further limit the growth of *C. hystrix*. Thus, N fertilizer should be added rather than P fertilizer in *C. hystrix* plantations to ensure their sustainable ecosystem function in the future.

4.2 Effects of increasing stand ages on ecosystem N dynamics

In the present study, it was found that increasing stand ages decreased the N concentration in leaves, which may have been due to N dilution as a result of enhanced *C. hystrix* growth (Cregger et al., 2014; Hayes et al., 2014). Additionally, lower litter N concentrations resulted in declining soil available N concentrations, which further decreased the N concentrations in leaves (Feng et al., 2018; Feng et al., 2019b). Higher C:N ratios in leaves and litter should be due to increasing C concentrations and decreasing N concentrations (Li et al., 2013).

Although higher litter inputs induced by increasing stand ages were found in the present study, reduced litter N concentrations might lead to declining soil available N concentrations in *C. hystrix* plantations (Chen et al., 2017; Feng et al., 2019b). In contrast to the findings of the present study, several studies reported that the soil available N concentrations were enhanced with increasing stand ages due to higher litter inputs and the accelerated decomposition of organic matter (Vitousek et al., 2010; Chen et al., 2017; Feng et al., 2019b). Higher PhOx, NAG, and CBH activities in the soil would help decompose organic matter to increase the soil available N concentrations (Lucas-Borja et al., 2016; Feng et al., 2017; Yang et al., 2021). MBN in the top layer was reduced with increasing stand ages, which may have been driven by declining litter N concentrations (Feng et al., 2019b). The reduced soil available N concentrations and MBN caused by increasing stand ages may not sustain the enhanced growth of *C. hystrix* (Feng et al., 2019a). The soil C:N and MBC:MBN ratios were enhanced with increasing stand ages, indicating that the mineralization rate of organic matter was reduced and that N in the soil might become a limiting element for plant growth (Yang et al., 2021). In contrast, previous studies have demonstrated that N may be not a limiting factor in subtropical forests due to the high level of N deposition (Reich and Oleksyn, 2004; Huang et al., 2013; Yang et al., 2021).

4.3 Effects of increasing stand ages on ecosystem P dynamics

Increasing stand ages increased the P concentrations in litter and sustained a stable P concentration in the leaves of *C. hystrix* plantations, which resulted in declining N:P ratios in litter and plants. Stable leaf P concentrations and increasing litter P concentrations in *C. hystrix* plantations under increasing stand ages led to decreased P resorption (Figure S1), and lower P resorption indicated that the leaf P concentrations met the growth requirements for *C. hystrix* (He et al., 2011; Du et al., 2020). Plant available P concentrations mainly result from the decomposition of organic P in tropical forests (Crews et al., 1995; Lie et al., 2019). The enhanced litter biomass and litter P concentrations caused by increasing stand ages lead to higher total P concentrations in the soil (Jara et al., 2009; Martinez-Garza et al., 2016), which led to stable P concentrations in the leaves of *C. hystrix* (Huang et al., 2013). In addition, *C. hystrix* requires less P than most other species in tropical China. Huang et al. (2012) measured 71 species in a monsoon evergreen broad-leaved forest that had been well protected for more than 400 years in southern China and found that the leaf P concentrations of most species were higher than that of *C. hystrix*.

In the present study, it was found that increased soil total P concentrations and MBP rather than plant P resorption provided a higher P supply to plants with increasing stand ages. Increased MBP in the top soil layer with increasing stand ages may be attributable to increased litter P concentrations (Zhao et al., 2009;

Bai et al., 2021). Lower AP activity in the deep layer (20–30 cm) suggested that P limitation was decreased under increasing stand ages in *C. hystrix* plantations (Feng et al., 2017). It was found that the soil available P concentrations in the top layer (0–10 cm) were lowered under increasing stand ages, which may be related to enhanced leaching (Tiecher et al., 2020) and P uptake by fine roots (Wang et al., 2021). In addition, reduced AP activity in *C. hystrix* plantations induced by increasing stand ages may contribute to decreased soil available P concentrations in the top layer (Wang et al., 2019). Many studies have found that soil total P concentrations are lower in older forests than in younger forests (Feng et al., 2017; Chen et al., 2018; Yang et al., 2021), which may be driven by higher P accumulation in plants as a result of increasing plant growth (Kitayama et al., 2000; Vitousek et al., 2010; See et al., 2015). P limitations in subtropical forests are exacerbated by increasing stand ages due to declining total and available P concentrations in the soil, as demonstrated by other studies (Kitayama et al., 2000; See et al., 2015). In contrast, the present study found that increasing stand ages led to higher total P concentrations in the soil of *C. hystrix* plantations, which may be related to the lower P requirement for maintaining the growth of *C. hystrix* (Huang et al., 2012). Crews et al. (1995) and Frizano et al. (2002) showed that soil P concentrations increased with stand ages, which might be associated with increasing P accumulation in plant biomass and due to litter returns to the soil (Vitousek et al., 2010).

5. Conclusions

Increasing stand ages reduced the N concentrations in litter, soil, microorganisms, and plants but increased their P concentrations, which led to lower N:P ratios in the *C. hystrix* plantation ecosystem in response to increasing stand ages. Overall, *C. hystrix* plantations become relatively N deficient over time, and their P limitation may be alleviated with increasing stand age in southern China. Based on the results of this study, the increase in potential N mineralization with increasing stand age did not meet the plant N demand, while the increases in total soil P and MBP facilitated the plant uptake of P. The shift in the nutrient balance under increasing stand age will increase the importance of N availability in subtropical plantation forest ecosystems as well as influence the availability of P.

Acknowledgments

This study was jointly funded by the Key Research and Development Program of Guangdong Province (Grant No. 2020B1111530004), the National Natural Science Foundation of China (Grant Nos. 41977287, 32101342 and 31971459), and the China Postdoctoral Science Foundation (Grant Nos. 2021M703259, 2021M703260 and 2021M693220). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

CRedit authorship contribution statement

Xu Li and **Ting Wu** : Conceptualization, Investigation, Formal analysis, Statistical analyses, Visualization, Writing—original draft. **Zhiyang Lie** and **Luis Carlos Ramos Aguila** : Writing—review & editing. **Yue Liu** , **Yan Cheng** and **Fen Jiang** : Investigation. **Xujun Liu** : Statistical analyses, Writing—review & editing. **Juxiu Liu** : Conceptualization, Writing—review & editing, Project administration, Funding acquisition, Supervision.

Statements & Declarations

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.

References

- Ali A , Hussain, M, Ali S, Akhtar K, Muhammad MW, Zamir A, Ali A, Nizami SM, Ahmad B, Harrison MT, Fahad S, Zhou ZX, Yi SJ (2020). Ecological stoichiometry in *Pinus massoniana* L. plantation: Increasing nutrient limitation in a 48-year chronosequence. *Forests*, 13, 469.
- Bai X, Dippold MA, An S, Wang B, Zhang H, Loeppmann S (2021). Extracellular enzyme activity and stoichiometry: The effect of soil microbial element limitation during leaf litter decomposition. *Ecological*

Indicators, 121.

Bonner MTL, Schmidt S, Shoo LP (2013). A meta-analytical global comparison of aboveground biomass accumulation between tropical secondary forests and monoculture plantations. *Forest Ecology and Management*, 291, 73–86.

Bremner JM, Mulvaney CS (1982). Nitrogen–total. In: *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*. Agronomy Monograph No. 9, 2nd edn (eds Page AL, Miller RH, Keeney DR), pp. 595–624. American Society of Agronomy, Madison, Wisconsin.

Bui EN, Henderson BL (2013). C:N:P stoichiometry in Australian soils with respect to vegetation and environmental factors. *Plant and Soil*, 373, 553–568.

Chen C, Park T, Wang X H, et al., (2019). China and India lead in greening of the world through land–use management. *Nature Sustainability*, 2, 122–129.

Chen HYH, Brant AN, Seedre M, Brassard BW, Taylor AR (2017). The contribution of litterfall to net primary production during secondary succession in the boreal forest. *Ecosystems*, 20, 830–844.

Chen LL, Deng Q, Yuan ZY, Mu XM, Kallenbach RL (2018). Age-related C:N:P stoichiometry in two plantation forests in the Loess Plateau of China. *Ecological Engineering*, 120, 14–222.

Cregger MA, McDowell NG, Pangle RE, Pockman WT (2014) The impact of precipitation change on nitrogen cycling in a semiarid ecosystem. *Functional Ecology*, 28, 1534–1544.

Crews TE, Kitayama K, Fownes JH, Riley RH, Herbert DA, Mueller-Dombois D, Vitousek PM (1995). Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology*, 76, 1407–1424.

Dong HY, Ge JF, Sun K, Wang BZ, Xue JM, Wakelin SA, Wu JS, Sheng WX, Liang CF, Xu QF, Jiang PK, Chen JH, Qin H (2021). Change in root-associated fungal communities affects soil enzymatic activities during *Pinus massoniana* forest development in subtropical China. *Forest Ecology and Management*, 482, 118817.

Deng L, Wang KB, Chen ML, Shangguan ZP, Sweeney S (2013). Soil organic carbon storage capacity positively related to forest succession on the Loess Plateau, China. *Catena*, 110, 1–7.

Du E, Terrer C, Pellegrini AFA, Ahlstrom A, van Lissa CJ, Zhao X, Xia N, Wu X, Jackson RB (2020). Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature Geoscience*, 13, 221–226.

Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, Enquist BJ (2010). Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist*, 186, 593–608.

Erinle K, Doolette OA, Marschner P (2019). Changes in phosphorus pools in the detritosphere induced by removal of P or switch of residues with low and high C/P ratio. *Biology and Fertility of Soils*, 56, 1–10.

Fan HB, Wu JP, Liu WF, Yuan YH, Hu L, Cai QK (2015). Linkages of plant and soil C:N:P stoichiometry and their relationships to forest growth in subtropical plantations. *Plant and Soil*, 392, 127–138.

Fang YT, Gundersen P, Mo JM, Zhu WX (2008). Input and output of dissolved organic and inorganic nitrogen in subtropical forests of South China under high air pollution. *Biogeosciences*, 5, 339–352.

FAO, 2020. Global forest resources assessment 2020—key findings[R/OL]. [2021–04–09]. <http://www.fao.org/3/CA8753EN/CA8753EN.pdf>.

Feng C, Ma Y, Fu SL, Chen HYH (2017). Soil carbon and nutrient dynamics following cessation of anthropogenic disturbances in degraded subtropical forests. *Land Degradation & Development*, 28, 2457–2467.

Feng C, Wang Z, Zhu Q, Fu SL, Chen HYH (2018). Rapid increases in fine root biomass and production following cessation of anthropogenic disturbances in degraded forests. *Land Degradation & Development*,

29, 461–470.

Feng C, Ma YH, Jin X, Wang Z, Ma Y, Fu SL, Chen HYH (2019a). Soil enzyme activities increase following restoration of degraded subtropical forests. *Geoderma*, 351, 180–187.

Feng C, Wang Z, Ma Y, Fu SL, Chen HYH (2019b). Increased litterfall contributes to carbon and nitrogen accumulation following cessation of anthropogenic disturbances in degraded forests. *Forest Ecology and Management*, 432, 832–839.

Frizano J, Johnson AH, Vann DR, Scatena FN (2002). Soil phosphorus fractionation during forest development on landslide scars in the Luquillo Mountains, Puerto Rico. *Biotropica*, 34, 17–26.

Gusewell S (2004). N:P ratios in terrestrial plants: Variation and functional significance. *New Phytologist*, 164, 243–266.

Hayes P, Turner BL, Lambers H, Laliberte E (2014). Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology*, 102, 396–410.

He YJ, Qin L, Li ZY, Liang XY, Shao MX, Tan L (2013). Carbon storage capacity of monoculture and mixed-species plantations in subtropical China. *Forest Ecology and Management*, 295, 193–198.

He H, Bleby MT, Veneklaas EJ, Lambers H (2011). Dinitrogen-fixing *Acacia* species from phosphorus-impooverished soils resorb leaf phosphorus efficiently. *Plant Cell & Environment*, 34, 2060–2070.

Hietz, P. et al (2011). Long-term change in the nitrogen cycle of tropical forests. *Science*, 334, 664–666.

Hua FY, Bruijnzeel L A, Meli P, Martin PA, Zhang J, Nakagawa S, Miao XR, Wang WY, McEvoy C, Pena-Arancibia JL, Brancalion PHS, Smith P, Edwards DP, Balmford A (2022). The biodiversity and ecosystem service contributions and trade-offs of forest restoration approaches. *Science*, abl4649.

Huang J, Zhang W, Zhu X, Gilliam FS, Chen H, Lu X (2015). Urbanization in China changes the composition and main sources of wet inorganic nitrogen deposition. *Environment Science Pollution Research*, 22, 6526–6534.

Huang WJ, Liu JX, Wang YP, Zhou GY, Han TF, Li Y (2013). Increasing phosphorus limitation along three successional forests in southern China. *Plant and Soil*, 364, 181–191.

Huang WJ, Zhou GY, Liu JX (2012). Nitrogen and phosphorus status and their influence on aboveground production under increasing nitrogen deposition in three successional forests. *Acta Oecologica*, 44, 20–27.

Jara P, Martinez E, Campo J (2009). N and P dynamics in the litter layer and soil of Mexican semi-arid forests, state of Morelos. *Agriculture, Ecosystems & Environment*, 130, 164–170.

Jenkinson DS, Brookes PC, Powlson DS (2004). Measuring soil microbial biomass. *Soil Biology & Biochemistry*, 36, 5–7.

Kitayama K, Majalap-Lee N, Aiba S (2000). Soil phosphorus fractionation and phosphorus-use efficiencies of tropical rainforests along altitudinal gradients of Mount Kinabalu, Borneo. *Oecologia*, 123, 342–349.

Li H, Li J, He Y, Li S, Liang Z, Peng C, Polle A, Luo ZB (2013). Changes in carbon, nutrients and stoichiometric relations under different soil depths, plant tissues and ages in black locust plantations. *Acta Physiologiae Plantarum*, 35, 2951–2964.

Lie ZY, Lin W, Huang WJ, Fang X, Huang CM, Wu T, Chu GW, Liu SZ, Meng Z, Zhou GY, Liu JX (2019). Warming changes soil N and P supplies in model tropical forests. *Biology and Fertility of Soils*, 55(7), 751–763.

Liu JX, Huang WJ, Zhou GY, Zhang DQ, Liu SZ, Li YY (2013). Nitrogen to phosphorus ratios of tree species in response to elevated carbon dioxide and nitrogen addition in subtropical forests. *Global Change*

Biology, 19, 208–216.

Liu JX, Liu SE, Li YY, Liu SZ, Yin GC, Huang J, Xu Y, Zhou GY (2017). Warming effects on the decomposition of two litter species in model subtropical forests. *Plant and Soil*, 420, 277–287.

Liu YY, Fang Y, An SS (2020). How C:N:P stoichiometry in soils and plants responds to succession in *Robinia pseudoacacia* forests on the Loess Plateau, China. *Forest Ecology and Management*. 475, 118394.

Liu D, Huang Y, Sun H, An S (2018). The restoration age of *Robinia pseudoacacia* plantation impacts soil microbial biomass and microbial community structure in the Loess Plateau. *Catena*, 165, 192–200.

Lucas-Borja ME, Hedo J, Cerda A, Candel-Perez D, Vinegla B (2016). Unravelling the importance of forest age stand and forest structure driving microbiological soil properties, enzymatic activities and soil nutrients content in Mediterranean Spanish black pine (*Pinus nigra* Ar. ssp. *salzmannii*) Forest. *Science of the Total Environment*, 562, 145–154.

Ma Z, Chen HYH, Bellingham P (2017). Effects of species diversity on fine root productivity increase with stand development and associated mechanisms in a boreal forest. *Journal of Ecology*, 105, 237–245.

Markewitz D, Figueiredo RdO, de Carvalho CJR, Davidson EA (2012). Soil and tree response to P fertilization in a secondary tropical forest supported by an Oxisol. *Biology and Fertility of Soils*, 48, 665–678.

Martinez-Garza C, Campo J, Ricker M, Tobon W (2016). Effect of initial soil properties on six-year growth of 15 tree species in tropical restoration plantings. *Ecology & Evolution* 6, 8686–8694.

Moreno-Mateos D, Barbier EB, Jones PC, Jones HP, Aronson J, Lopez-Lopez JA, McCrackin ML, Meli P, Montoya D, Rey Benayas JM (2017). Anthropogenic ecosystem disturbance and the recovery debt. *Nature Communications*, 8, 14163.

Mori T (2020). Does ecoenzymatic stoichiometry really determine microbial nutrient limitations? *Soil Biology & Biochemistry*, 146.

Morrison E, Newman S, Bae HS, He Z, Zhou J, Reddy KR, Ogram A (2016). Microbial genetic and enzymatic responses to an anthropogenic phosphorus gradient within a subtropical peatland. *Geoderma*, 268, 119–127.

Nelson DW, Sommers LE (1982). Carbon and organic matter. In: Page AL, Mille RH, Keeney DR (eds) *Methods of soil analysis—Part 2: chemical and microbiological properties*. American Society of Agronomy, Madison, pp. 561–579.

Paul KI, Polglase PJ, Nyakuengama JG, Khanna PK (2002). Change in soil carbon following afforestation. *Forest Ecology and Management*, 168, 241–257.

Reich PB, Oleksyn J (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 11001–11006.

See CR, Yanai RD, Fisk MC, Vadeboncoeur MA, Quintero BA, Fahey TJ (2015). Soil nitrogen affects phosphorus recycling: foliar resorption and plant–soil feedbacks in a northern hardwood forest. *Ecology*, 96, 2488–2498.

Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) *Methods of soil analysis. Part 2. Microbiological and biochemical properties*, SSSA Book Ser, vol 5. SSSA, Madison, pp 801–834.

Tiecher TL, Lourenzi CR, Giroto E, Tiecher T, De Conti L, Marques ACR, Silva LOS, Marchezan C, Brunetto G, Ceretta CA (2020). Phosphorus forms leached in a sandy Typic Hapludalf soil under no-tillage with successive pig slurry applications. *Agricultural Water Management*, 242.

Vance E, Brookes P, Jenkinson D (1987), An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry*, 19, 703–707.

- Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010). Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, 20, 5–15.
- Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010). The use of chronosequences in studies of ecological succession and soil development. *Journal of Ecology*, 98, 725–736.
- Wang CQ, Xue L, Jiao RZ (2019). Soil organic carbon fractions, C–cycling associated hydrolytic enzymes, and microbial carbon metabolism vary with stand age in *Cunninghamia lanceolata* (Lamb.) Hook plantations. *Forest Ecology and Management*, 482, 118887.
- Wang ZF, Zheng FL (2021). Impact of vegetation succession on leaf-litter-soil C:N:P stoichiometry and their intrinsic relationship in the Ziwuling Area of China's Loess Plateau. *Journal of Forest Research*, 32, 697–711.
- Wang L, Liu Y, Zhu X, Zhang Y, Yang H, Dobbie S, Zhang X, Deng A, Qian H, Zhang W (2021). Effects of arbuscular mycorrhizal fungi on crop growth and soil N₂O emissions in the legume system. *Agriculture, Ecosystems & Environment*, 322.
- Wang RZ, Lu LY, Creamer CA, Dijkstra FA, Liu HY, Feng X, Yu GQ, Han XG, Jiang Y (2017). Alteration of soil carbon and nitrogen pools and enzyme activities as affected by increased soil coarseness. *Biogeosciences*, 14, 2155–2166.
- Waterworth R, Raison RJ, Brack C, Benson M, Khanna P, Paul K (2007). Effects of irrigation and N fertilization on growth and structure of *Pinus radiata* stands between 10 and 29 years of age. *Forest Ecology and Management*, 239, 169–181.
- Xu HC, Zheng L, Wang HX, Nong Y, Chen YK, Li M, Cai DX, You YM (2020). Seedling regeneration and spatial correlation between seedlings and seed trees in plantation by large diameter wood cultivation in south subtropical China. *Chinese Journal of Applied Ecology*, 31(4), 1055–1062. (in Chinese)
- Yan Z, Kim N, Han W, Guo Y, Han T, Du E, Fang J (2014). Effects of nitrogen and phosphorus supply on growth rate, leaf stoichiometry, and nutrient resorption of *Arabidopsis thaliana*. *Plant and Soil*, 388, 147–155.
- Yang B, Qi KB, Bhusal DR, Huang JS, Chen WJ, Wu QS, Hussain A, Pang XY (2020). Soil microbial community and enzymatic activity in soil particle-size fractions of spruce plantation and secondary birch forest. *European Journal of Soil Biology*, 99, 103196.
- Yang SB, Feng C, Ma YH, Wang WJ, Huang C, Qi CJ, Fu SL, Chen HYH (2021). Transition from N to P limited soil nutrients over time since restoration in degraded subtropical broadleaved mixed forests. *Forest Ecology and Management*, 494, 119298.
- Yu GR, Chen Z, Piao SL, Peng CH, Ciais P, Wang QF, Li XR, Zhu XJ (2014). High carbon dioxide uptake by subtropical forest ecosystems in the East Asian monsoon region. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 4910–4915.
- Yuan ZY, Chen HYH (2012). A global analysis of fine root production as affected by soil nitrogen and phosphorus. *Proceedings of the Royal Society B: Biological Sciences*, 279, 3796–3802.
- Zhang C, Liu GB, Xue S, Sun CL (2013). Soil organic carbon and total nitrogen storage as affected by land use in a small watershed of the Loess Plateau, China. *European Journal of Soil Biology*, 54, 16–24.
- Zhang H, Sun M, Wen Y, Tong R, Wang G, Wu Q, Li Y, Wu T (2022). The effects of stand age on leaf N:P cannot be neglected: A global synthesis. *Forest Ecology and Management*, 518.
- Zhao Q, Zeng DH, Fan ZP, Yu ZY, Hu YL, Zhang J (2009). Seasonal variations in phosphorus fractions in semiarid sandy soils under different vegetation types. *Forest Ecology and Management*, 258, 1376–1382.

Zhou GY, Peng CH, Li YY, Liu SZ, Zhang QM, Tang XL, Liu JX, Yan JH, Zhang DQ, Chu GW (2013). A climate change-induced threat to the ecological resilience of a subtropical monsoon evergreen broad-leaved forest in Southern China. *Global Change Biology*, 19, 1197–1210.

Zhou GY, Yin GC, Tang XL, Wen DZ, Liu CP, Kuang YW, Wang WT (2018). Carbon storage of forest ecosystems in China: Biomass Equation. Beijing: Science Press.

Hosted file

figures.docx available at <https://authorea.com/users/633971/articles/652037-increasing-stand-age-increases-n-deficiency-but-alleviates-relative-p-limitations-in-castanopsis-hystrix-plantations-in-southern-china>

Hosted file

Tables.docx available at <https://authorea.com/users/633971/articles/652037-increasing-stand-age-increases-n-deficiency-but-alleviates-relative-p-limitations-in-castanopsis-hystrix-plantations-in-southern-china>