

Determining the Necessity of Applying N and P Fertilizer in a Mature Subtropical *Torreya Grandis* Orchard

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Research

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Abstract

Background In managed orchards, fertilizer treatments facilitate both high productivity and environmental pollution. Because economic profit takes priority over environmental cost, increasing amounts of chemical nitrogen and phosphorus fertilizer have been used in mature subtropical *Torreya grandis* orchards. However, given the magnitude of global nitrogen deposition, it's worth considering whether heavy fertilizer treatment is necessary.

Methods To elucidate the balance between *T. grandis* nutrient demands and the fertilizer supply, we determined the C, N, and P foliar and soil concentrations ([C], [N], [P]) at five orchards undergoing long-term varied intensity fertilizer treatments.

Results After documenting the dynamic variation of available plant nutrients and the corresponding resorption efficiency, we found that increasing the fertilizer supply elevated foliar [P], yet foliar [C] and [N] remained stable. Because *T. grandis* was already equipped with a high nutrient content, the increased foliar [P] levels decreased C:P and N:P ratios. These results demonstrate that extra fertilizer in the N-saturated environment disturbs P-limitation. Furthermore, we also found that fertilizer supply failed to improve carbon accumulation, which in addition to soil nutrient content and leaf [P], highly impacted productivity.

Conclusions Thus, based on the results of this study, there are ample reasons to propose rejecting N addition in the present orchards, and we recommend organic management as a more conducive method to realize sustainable development.

Introduction

In order to facilitate a rapid increase in soil nutrients and guarantee profitable productivity, escalating amounts of chemical nitrogen (N) and phosphorus (P) rich fertilizer have been applied in *T. grandis* orchards, without any scientific management guidance. N and P are key nutrients that play pivotal roles in controlling plant growth and litter decomposition as well as the ecosystems' biochemical cycles (Finzi et al. 2011; Penuelas et al. 2013; You et al. 2018). However, indiscriminate use of chemical fertilizers has caused abnormally high concentrations of N and P to accumulate in the soil, which has severely stressed the terrestrial plants' physiological processes. Furthermore, the excessive use of chemical fertilizer has also generated serious adverse environmental consequences, such as non-point source pollution (Sun et al. 2019) and N induced soil acidification (especially due to NH_4^+), both of which have been observed in multiple ecosystems (Fernández-Escobar et al. 2009; Zhao and Zeng 2019; Zhu and Chen 2002). It should be noted that soil acidification further changes ecosystem biogeochemistry, which increases cationic nutrient leaching and thereby reduces plant productivity (Dai et al. 2017; Zhang et al. 2016).

In the field conditions, N input to an N-limited ecosystem, such as boreal forests, will improve net primary productivity (NPP) through a direct fertilizing effect on vegetation (Lebauer and Treseder 2008a). as a

Loading [MathJax]/jax/output/CommonHTML/jax.js in many regions of the world (i.e., United States, western

Europe, and China) currently exceeds $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, especially in tropical and subtropical areas (Liu et al. 2013; Meunier et al. 2016), where over $80\text{--}120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ has been reported (Yu et al. 2016). Therefore, as N deposition levels continue to accelerate, N-limitation has been subsequently alleviated (Tao and Hunter 2012), and there is a high likelihood that the ecosystem is shifting to an N-enriched status (Yu et al. 2018). N deposition has also changed the soil stoichiometry by accelerating the soil P cycle in tropical and subtropical forests. Specifically, excessive N in temperate forests has been shown to alter the biogeochemical cycles of essential plant nutrients (Ferretti et al. 2015; Gilliam et al. 2016; Sardans et al. 2016a), but it also introduced P-limitation in forest ecosystems (Du et al. 2020; Du et al. 2016; Güsewell 2004; Vitousek et al. 2010). Fang et al. (2019) reported N saturation in three subtropical sites and noted that P deficiency is becoming progressively more problematic. Anthropogenic alternation of regional P and N cycling has led to large areas of southern China forests transmuting due to human-induced P-limitation (Du et al. 2016). Thus, NPP has transformed from being N limited to P limited in many forest ecosystems (LeBauer and Treseder 2008b).

Under intensive management, long-term addition of balanced compound fertilizer (e.g., N:P:K = 15:15:15) may cause excess P in orchards, as plants generally require less P than N (Macy 1936). P is an essential element for nucleic acids and membrane lipids. Although P sensing and signaling are not fully understood, there appears to be a series of physiological processes in plants that are either stimulated or suppressed in response to P supply (Fang et al. 2009). Unlike the immobile N in the plant cell wall, most leaf P is hydrolyzable and, therefore, more easily resorbed (Ågren 2008; McGroddy et al. 2004). In fact, due to its indiscriminate uptake, greater variability of foliar P has been reported even if it's not needed at that growth stage (Ostertag 2010). Species in P-poor environments, such as subtropical evergreen trees, are equipped with a corresponding adaptation mechanism, which makes them more susceptible to toxic eutrophication under excessive P addition (Musick 1978).

Soil N and P availability when combining with N deposition and/or external addition can influence forest productivity and ecosystem processes (Elser et al. 2000; Finzi et al. 2011). Trees may keep leaf nutrient concentrations and their ratios stable by modulating the nutrients coming from branches, roots or senescent leaves (Cernusak et al. 2010; Yan et al. 2016). Recently, foliar N and P concentration stoichiometry ratios (i.e., N/P, C/N, C/P) have been used to indicate soil N- and/or P-limitations to plant growth (Aerts and Chapin III 1999; Güsewell 2004; Koerselman and Meuleman 1996). In addition, nutrient resorption [nitrogen (NRE) and/or phosphorus (PRE)] from senescing plant tissues and the proficiency of nutrient conservation (Lin et al. 2010) are also widely used as indicators in studies of nutrient cycling between the plants and soil in fluctuating environments. Generally, nutrients would transfer from senescent leaf to trunk before falling off, thereby maintaining the plant nutrients at a favorable level. The NRE and PRE might depend on the type of nutrient limitation (Güsewell 2005) and vary in response to the plant's intrinsic genetic characteristics (Sadanandan Nambiar and Fife 1991; Silla and Escudero 2003). NRE/PRE is commonly employed to determine the relative limitation between N and P; as NRE/PRE values > 1 imply a stronger N-limitation at the ecosystem scale (Du et al. 2020). However, previous studies

predominantly focused on the effect of single N or P addition on leaf nutrients or resorption; thus, the effect of intensive P addition on PRE in subtropical forests is not well understood.

Due to the high economic value of its nuts, *T. grandis* has become one of the most important commercial tree species in southeast China. During the past three decades, the planting area of *T. grandis* has steadily increased. As a result, farmers are facing escalating economic pressure, which has led to excessive fertilizer consumption, and ultimately deterioration of the soil's physical and chemical properties. Thus, it is critical to determine the optimal amount of fertilizer necessary to achieve ideal growth. A comprehensive understanding of fertilizer impact on crop quantity and soil quality is critical for improving fertilizer treatment strategies in economic considerations and maintaining a healthy soil environment. To solve the above problems, we examined the C, N, and P stoichiometry of soil as well as green and senescent leaf of a mature *T. grandis*, in five orchards plots with varying fertilizer treatments. The objective of this study was to: 1) evaluate whether the continuously increasing N deposition in subtropical forests has alleviated the N-limitation, making P the limiting factor restricting plant growth; (2) determine the optimal fertilizer supply; and (3) assess whether large amounts of fertilizer, i.e., N and/or P, negatively impacts the plants. These results are expected to provide fertilization guidelines and recommendations to help farmers reduce costs and soil pollution, while ensuring optimal production.

Materials And Methods

Study site

The study was conducted at the origination locale of *T. grandis* – Chinese Torreya Forest Park (29.69-29.73°N, 120.49-51°E), in Zhuji city, Zhejiang Province, China. The physical and chemical properties of the soil in the study area consisted of: organic carbon (OC) – 18.2 g/kg; total N (TN) – 1.67 g/kg; total P (TP) – 1.56; hydrologic N (HN) – 132 mg/kg; and Olmes-P (AP) – 225 mg/kg. The soil was acidic with a pH = 3.67 ± 0.12 and was characterized as Hapludult soil type with respect to Soil Taxonomy (Gong et al. 2007). *T. grandis* trees of 130 years old growing in orchards with a density of 22–55 trees per hectare were selected as the study object. The average tree height was 7.8 ± 0.5 m (mean \pm SD), the diameter at breast height (DBH) was 46.2 ± 2.2 cm, the average crown breadth or trees was $6.0 \pm 0.6 \times 7.0 \pm 0.5$ m.

Nutrients addition

Fertilization was applied in each study orchard during the growing season. Different amounts of compound fertilizer with an N: P₂O₅: K₂O proportion ratio of 15:15:15 (a N: P: K content ratio of 1:0.44:0.83) were used as the nutrient addition treatments. The experimental design included five treatments and three replicates, totaling 15 100 m x 100 m plots. The control group was designated “F0”, which received no fertilization. The four nutrient addition treatments were designated as F1-F4, forming an increasing fertilization gradient. Each plot had 12–15 mature *T. grandis* plants, and were subjected to the same management practices except for nutrient addition. Fertilizer treatments were detailed in

Table 1, and fertilization was applied twice, each in April and July, at a depth of 10 cm below the soil's surface. Organic fertilizer was applied in plots F1, F2, and F4, in order to provide more organic matter.

Table 1
Fertilizer treatments and nutrients addition intensity in this study (CF: compound fertilizer; OF: organic fertilizer)

Fertilizer treatments	F0	F1	F2	F3	F4
	CK	Low	Medium	Medium	High
CF (kg/hm ²)	0	120	240	320	480
OF (kg/hm ²)	0	40	80	0	160
N (kg/hm ²)	0	18	36	48	72
P (kg/hm ²)	0	7.9	15.8	21.1	31.7
N _i (%)	7.9	7.6	8	7.5	8.9
P _i (%)	14.4	29.3	20.7	11.6	24.3

Sample collection and measurements

Soil samples were collected at the same time when leaf samples were collected. Each soil sample was a mixture of three soil collections from a 0–20 cm depth, and each location was randomly selected along the diagonal of each plot. The soil was dried and sifted to measure the particle size distribution and other soil chemical properties. In each plot, **three green leaf sample groups (30 leaves for each group)** were collected from the apicous healthy shoots of random plants in the middle of the canopy. Similarly, three 1 m x 1 m **litter** collection boxes were arranged along the diagonal of each plot. Both sample sets - the green leaf samples and the mixed litter samples were individually oven-dried at 60 °C to a constant weight, then they were respectively ground up and sieved through a 1 mm mesh screen for further analyses.

The foliar and soil nutrient properties (i.e., Total N, Total P, hydrolysable N, Olsen-P, and soil organic carbon) as well as soil pH were measured in accordance with national forestry industry standards, as shown in Table 2.

Table 2
Measurement standard referred in this study

Standard	Administration
LY/T 1237–1999	National Forestry and Grassland Administration, 1999
LY/T 1239–1999	National Forestry and Grassland Administration, 1999
LY/T 1271–1999	National Forestry and Grassland Administration, 1999
LY/T 1228–2015	National Forestry and Grassland Administration, 2015
LY/T 1232–2015	National Forestry and Grassland Administration, 2015

Data analysis

To examine the relative nutrient limitation among the treatments, we calculated nutrient resorption efficiency (NuRE) using the following equation:

$$NuRE = \left[1 - \frac{Nu_{senescent}}{Nu_{green}} \times MLCF \right] \times 100\%$$

where, Nu_{green} and $Nu_{senescent}$ are the nutrient concentrations ([N], [P]) in green and senesced leaves, respectively, and MLCF is the mass loss correction factor with a value of 0.780 for evergreen species (Vergutz et al. 2012).

Given the large data set collected, we also tested the relationships between log-transformed nutrient stoichiometry of green and senescent leaves, by applying a type II linear regression model (SMA, standardized major axis; $Y \sim X$) using the `lmodel2` package in R (3.6.1). Y is the [C], [N], [P] or stoichiometry ratios in the foliar samples and X is the related variables in the senescent leaves; slope > 1 indicated dependence of Y variation on X, slope < 1 indicated an independence of Y on X, and slope = 1 indicated a synchronous change of X and Y.

Significant difference of each dependent variable (C, N, P stoichiometry of soil, foliar and nutrient resorption) among fertilizer treatments were tested by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) and Tamhane's T2. Distance correlation analysis was performed to assess the nutrient content correlation among the soil, green leaves, and senescent leaves. Results were considered significant when $P < 0.05$. All statistical analysis was performed using the SPSS software (version 20.0, SPSS Inc., Chicago, USA) and R (3.6.1).

Results

Effects of fertilization on soil nutrient characteristics

Fertilization treatments significantly affected soil chemical properties. Soil nutrient stoichiometry showed a strong positive correlation with the nutrient addition gradient (Fig. 1). Concentrations were ranged from 18.17 ~ 34.17 g/kg (OC), 1.67 ~ 3.38 g/kg (TN), 1.56 ~ 4.45 mg/kg (TP), 132 ~ 299 mg/kg (HN), and 225 ~ 1081.00 mg/kg (AP) depending on the specific treatment. Compared to the control, adding fertilizer increased the soil's TN, TP, HN, and Olsen-P by a maximum of 102%, 185%, 127%, and 380%, respectively. The addition of organic fertilizer raised the soil pH in samples F1, F2, and F4 above that of F0 and F3. Yet, by comparing samples F0, F2, and F3, it is apparent that organic fertilizer had no significant effect on soil organic carbon (SOC) content. Finally, as the fertilizer concentration increased, the soil N:P and C:P significantly decreased (Figs. 1g, h), but C:N (11.00 ± 0.87) remained stable throughout the four experimental treatments.

Effects of fertilizer on foliar nutrient characteristics

Fertilizer treatment increased foliar nutrient contents, which depended on the nutrient element and growing status (Fig. 2). Generally, green leaf uniformly exhibited a higher nutrient concentration than senesced leaf (Fig. 2), but different nutrient elements, [C], [N], and [P], showed different patterns. For green leaf, although [N] and [C] tended to be the highest under medium fertilizer treatments, there was little difference in nutrient contents in response to the different fertilizer supply intensities. In contrast, [P] exponentially increased with the fertilizer supply gradient (F2-F4) and tended to maximize under the high level fertilizer treatment (F4). For senescent leaf [C], [N], and [P], the resorption proficiency trend paralleled with that of the green leaf, although the magnitudes differed. Compared to the control, high level fertilizer supply increased [P] by 102% and 57% of green and senescent leaves, respectively. Similar to green leaf, there was no significant difference in [C] and [N] among different treatments in senescent leaf. Senescent leaf [P] under high level fertilizer supply (F3 and F4) was slightly higher than that of the other treatments. Green leaf [P] was significantly correlated with soil inorganic nitrogen concentration (indicated by soil HN) rather than inorganic phosphorus (indicated by soil Olsen-P) (Table 3), while there was no correlation of leaf [N] with either soil P or N content.

Table 3
Pearson correlation coefficients of the nutrients in leaves and soil

	Soil TN	Soil TP	Soil HN	Soil Olsen-P	SOC
Leaf P	0.789**	0.809**	0.906*	0.541	0.691**
Leaf C:P	-0.564*	-0.548*	-0.917*	-0.507	-0.476
Leaf N:P	-0.582*	-0.549*	-0.900*	-0.488	-0.512
** and * indicate statistical significances at α 0.01 and 0.05					

The C:N ratio of both green (2.15 ± 0.05) and senescent (2.47 ± 0.09) leaves showed no apparent change
 Loading [MathJax]/jax/output/CommonHTML/jax.js nt increase in [P] led to big variations of N:P and C:P in green

and senescent leaves, which declined with increasing fertilizer addition, excluding the control group. The highest nutrient utilization efficiency indicated by C:P and C: N were 27.92 (F1) and 2.21 (F1) in the green leaf. In addition, there was no correlation between foliar N or P with soil pH values.

Discussion

Effect of fertilizer on soil condition

Long-term N deposition and fertilization could elevate soil nutrient concentration beyond the demand of plant, altering the soil nutrient cycles (Huang et al. 2007). Because most soils in subtropical areas is severely acidic, improper and excessive use of fertilizers could further deteriorate soil physical, chemical, and biological properties, especially aggravating soil acidification and soil hardening. Decreases in soil pH caused by fertilizers are likely to reduce soil microbial and enzyme activities, which subsequently reduces organic matter mineralization and ultimately reduces “chemical facilitation” (Yang 2018). In this study, adding organic fertilizer effectively improved soil acidification (Fig. 1) and validated previous reports that organic fertilizer addition plays a positive role in soil acidification improvement in subtropical forests (Fang et al. 2019; Pang et al. 2019; Wang et al. 2014). However, unlike other studies, the improvement in our experiment was not related to the amount of organic fertilizer, but instead, it was negatively related to the amount of compound fertilizer (Chai et al. 2019). It is generally accepted that organic matters can increase available soil nutrients, which, however, was not significant in our study. The possible cause was attributed to the form of added N (NH_4NO_3), which lowered the positive priming effect of carbon inputs on mineralization of organic matters in soil (Chen et al. 2018).

Effect of fertilizer on plant C, N, and P stoichiometry

The primary goal of this research was to evaluate whether *T. grandis* requires fertilization or whether adding additional nutrients through fertilization can improve tree nutrition. In the present study, we found that fertilization tended to increase the green and senescent leaves [C], [N], and [P], but only the increase in [P] was statistically significant (Fig. 2). Furthermore, the average values of [N] and [P] were clearly higher than the corresponding values of global flora (Han et al. 2005; Reich and Oleksyn 2004) and most woody species in southeast China (Wu et al. 2012). Plants reduce nutrient resorption to the largest extent when the environmental nutrient availability exceeds their demands (Wright and Westoby 2003). The fact that foliar [N] remained stable, despite the increased nutrient supply, may indicate diminished N-limitation to plant growth, which can be explained by a plant behavior developed from adjusting to nutrient abundant environments. Only leaf [P] showed a significant increase under increasing fertilizer supply, resulting in a distinct decrease in C:P and N:P ratios. Therefore, our findings support our first hypothesis that long-term fertilization, combined with increasing nitrogen deposition, results in a N-enriched and P-limited environment. This result also confirms that N-limitation has been generally alleviated in the subtropical forest; while P-limitation in the study area was aggravated by N enrichment (Han et al. 2005;

The soil nutrients pool (TN, TP, HN, and SOC), under long-term fertilizer treatment, was much higher than that in other subtropical areas (Kou et al. 2016; Zhang et al. 2017). Long-term soil nutrient enrichment resulted in much higher *T. grandis* foliar nutrient (N and P) contents (Tang et al. 2018a) than other subtropical evergreen trees (Yan En-Rong et al. 2010); the average N and P values of 753 terrestrial species were 18.6 and 1.21 g/kg, respectively (Han et al. 2005). Due to the different N and P utility patterns in physiological process, regardless of species and site fertilizer supply, plants are able to store a greater percentage of inorganic P than N. Thus, following fertilizer treatment, foliage accumulated more P than N (Ostertag 2010). It was also observed that some plant species growing in P-limited environments might not downregulate P uptake when a higher supply of P is available (Ostertag 2010; Shane and Lambers 2007; Standish et al. 2007). When the environment P supply shifts from P-limited to non-limited condition, the plants may undergo an excessive P uptake, even to saturated or toxic levels. Hence, foliar [P] usually displays a much higher variation after fertilizer treatment. Therefore, it is not surprised that the foliar [P] nearly doubled after a high fertilizer treatment compared to the control group (Fig. 2). Given the generally low bioavailability of P in subtropical soils, *T. grandis* may have developed as an efficient mechanism to take up and accumulate P in response to the strong selective pressure (Chapin III et al. 1990; Ingestad 1974; Mulligan and Sands 1988). Unlike previous studies (Huang et al. 2016; Sardans et al. 2016b; Zhang et al. 2019), leaf [N] of *T. grandis* didn't change much in response to the increasing soil [N] availability, which implied that fertilizer treatments had no effect on *T. grandis* N absorption (Figs. 1 and 2). In this scenario, relatively stable leaf [C] and [N], but increased leaf [P] led to opposite trends between C:P and N:P and soil nutrients (Fig. 1; Table 3).

Stoichiometry homeostasis was used to analyze plant composition, ecosystem function, and nutrient limitation, especially for key elements such as C, N, and P ratios (Allen and Gillooly 2009; Hessen et al. 2004). Generally, plants uptake nutrients in appropriate proportions to maintain nutritional balance, depending on physiological consumption (Phoenix et al. 2012; Sardans et al. 2016b). The inherently higher variability of P concentration in plants, relative to N, was illustrated in a survey of European wetlands (Güsewell and Koerselman 2002). Similarly, our findings demonstrated that the correlations between foliar [P], [C], [P], and [N] were decoupled due to a more sensitive response of foliar [P] to fertilizer supply (Figs. 2 and 3). This suggests that the balance of biogeochemical C, N, and P cycles were broken by the fertilizer treatments, which could decrease ecosystem stability (Demars and Edwards 2007) and alter the N and P cycles (Yuan and Chen 2015), leading to degenerative feedback between the plant and ecosystem (Sistla and Schimel 2012). Under these circumstances, foliar [P] or the N:P ratio may not reflect the actual demands of the plant under P addition conditions (Greenwood et al. 2008). Although many studies indicate that the N:P ratio of mature leaves have been widely used to diagnose plant growth nutrient limitations, with thresholds (such as 10 and 20) to classify the plants into N-limited, N and P co-limited, and P limited plants (Güsewell 2004; Rejmánková 2005; Xu et al. 2017).

Furthermore, contrary to previous research (Li et al. 2016), fertilizer treatment in this study also failed to increase the aboveground biomass (indicated by a stable carbon sequestration) of *T. grandis*. Moreover, the fertilizer significantly reduced the C:P (indicating the P utilization efficiency in productivity) of *T.*

Loading [MathJax]/jax/output/CommonHTML/jax.js utilization efficiency of plants was reduced by the increased

nutrient supply (Dijkstra et al. 2016). Our analysis suggested that both P and N fertilizer might be unnecessary for *T. grandis*.

Effect of fertilizer on plant nutrient resorption

Table 4
Relationships of nutrients among soil, leaves, and litters through SMA analyses

Y	X	Slope	Intercept	R^2
Leaf N	soil TP	0.24	1.26	0.63*
Leaf N	Soil HN	0.21	0.87	0.48*
Litter N	Leaf N	0.9	0.25	0.72**
Litter P	Soil TP	0.69	-0.02	0.59*
Litter C	Leaf C	1.63	-1.12	0.51*
Leaf C	SOC	0.18	1.42	0.88**
Leaf C:P	Leaf P	-1.03	1.70	0.99**
Leaf C:P	Leaf P	-1.02	1.36	0.98**
PRE	Leaf P	-1.02	1.36	0.98**
* and ** are 2-tail sig. $P < 0.1$ and 0.05				

Acquisition (root uptake) and conservation (resorption from senescent tissue) are two important biological strategies for plants to maintain balanced nutrition where soil nutrition is deficient. These processes are also important in cycling nutrients between the soil and plants (Killingbeck 1986). Choosing between alternative strategies for plants depends on the cost (time and energy) of each process and the species characteristics. Short-term experiments have demonstrated that generally, plants will reduce nutrient utilization efficiency when the availability of that nutrient increases in the plant's natural environment (Yuan and Chen 2015). While the relationship between foliar resorption and soil nutrient concentration is not consistent (Aerts 1996; Aerts and Chapin III 1999; Yuan 2015). Our results indicated a constant NRE, which further verified our supposition concerning N enrichment in the orchard (Fig. 4a). However, increased P migration from senescent leaf to green leaf (PRE) in response to increasing fertilizer supply conflicts with the negative correlation between fertilizer treatment and nutrient resorption (Yuan and Chen 2015). This counterintuitive result from a single-resource conservation standpoint has been observed in previous studies (Boerner 1986; Sabaté et al. 1995); yet multiple-element theory much appropriately explains variability in foliar nutrient resorption (See et al. 2015). Based on a Pearson correlation analysis, we found that green leaf [P] was not related to soil [Olsen-P], but it was highly correlated with soil N content (Table 3). It should be noted that the root system can directly uptake

inorganic nutrients (Olsen-P and hydrolysable nitrogen) and therefore plays a more important role in nutrient absorption than organic nutrients. This inorganic nutrient preference may help explain the absence of a common trend in studies comparing P resorption to soil P availability (Aerts 1996; Aerts and Chapin III 1999). Under the circumstances, although the soil and leaves were characterized by a higher [P] compared to other studies of evergreen trees (Tang et al. 2018b), N enrichment was responsible for the amplified P-limitation (Fig. 4b), which subsequently elevated leaf P uptake (Agüero et al. 2014; Tian et al. 2019). The standardized major axis (SMA) analysis showed that leaf [P] is independent of soil [P], which supports the above hypothesis. Strong dependencies, indicated by a slope > 1 , were found only between senescent [C] and leaf [C] (Table 4). For an evergreen species with a long leaf life span, *T. grandis* exhibited a more conservative P use strategy as opposed to the external root uptake strategy, regardless of soil P availability. Similar results were also reported in some non-mycorrhizal species (Laliberté et al. 2012). This mechanism allows plants to maintain a higher nutrient absorption efficiency with relatively less cost (energy and time) but lowers the utilization efficiency and productivity (Wright and Westoby 2003).

Conclusion

As N deposition and long-term fertilizer treatment intensified, there was a significant increase in the soil N and P, which improved leaf N and P concentrations. In our study area, N enrichment was indicated by stable and decoupling relationships between the soil and leaf [N]. Although *T. grandis* has a high level of leaf [N] and [P], N enrichment amplified the physiological P limitation (deficiency). Unfortunately, the present fertilizer modes did not seem to enhance productivity (C fixation). Excessive fertilizer application both wastes resources and negatively impacts nutrient uptake and soil physicochemical properties. Thus, we recommended rejecting N fertilizer addition and increasing application of organic fertilizer as needed. Although P-limitation was demonstrated in this study area, whether the P was deficient in the soil requires further exploration through a single-P element control experiment.

Abbreviation

Abbreviations	Definitions
<i>T. grandis</i>	<i>Torreya grandis</i>
C	Carbon
N	Nitrogen
P	phosphorus
[C]	Carbon concentration
[N]	Nitrogen concentration
[P]	Phosphorus concentration
NPP	Net primary productivity
NRE	Nitrogen resorption efficiency
PRE	Phosphorus resorption efficiency
RR	Relative nutrient resorption

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All the authors listed have contributed significantly to this research work and approved to submit this manuscript to your journal. Specifically, Dr. Yini Han, conducted the data analysis, and data interpretation and drafted the manuscript; Dr. Songheng Jin instructed the field experiment design, data interpretation; Dr. Geofeng Gao and Tonggui Wu helped develop ideas and revised the manuscript; Dr. Wenjing Chen and postgraduate Yongliang Ji helped in field experiment design and data collected.

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References

1. Administration NFaG (1999a) LY/T 1237-1999. China Standards Press, Beijing
2. Administration NFaG (1999b) LY/T 1239-1999. China Standards Press, Beijing
3. Administration NFaG (1999c) LY/T 1271-1999. China Standards Press, Beijing
4. Administration NFaG (2015a) LY/T 1228-2015. China Standards Press, Beijing
5. Administration NFaG (2015b) LY/T 1232-2015. China Standards Press, Beijing
6. Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology*:597-608
7. Aerts R, Chapin III FS (1999) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. In: *Advances in ecological research*, vol 30. Elsevier, pp 1-67
8. Agüero ML, Puntieri J, Mazzarino MJ, Grosfeld J, Barroetaveña C (2014) Seedling response of *Nothofagus* species to N and P: linking plant architecture to N/P ratio and resorption proficiency *Trees* 28:1185-1195
9. Ågren GI (2008) Stoichiometry and nutrition of plant growth in natural communities *Annual review of ecology, evolution, and systematics* 39:153-170
10. Allen AP, Gillooly JF (2009) Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling *Ecology Letters* 12:369-384
11. Boerner RE (1986) Seasonal nutrient dynamics, nutrient resorption, and mycorrhizal infection intensity of two perennial forest herbs *American Journal of Botany* 73:1249-1257

12. Cernusak LA, Winter K, Turner BL (2010) Leaf nitrogen to phosphorus ratios of tropical trees: experimental assessment of physiological and environmental controls *New Phytologist* 185:770-779 doi:10.1111/j.1469-8137.2009.03106.x
13. Chai B, Lv J, Li Q, Wu J, Song X (2019) Interaction of Biochar Amendment and Nitrogen Deposition on Soil Microbial Biomass Carbon and Enzyme Activity in a *Torreya grandis* Orchard *Polish Journal of Environmental Studies* 28 doi:10.15244/pjoes/95035
14. Chapin III FS, Schulze E, Mooney HA (1990) The ecology and economics of storage in plants *Annual review of ecology and systematics* 21:423-447
15. Chen S et al. (2018) Plant diversity enhances productivity and soil carbon storage *Proceedings of the National Academy of Sciences of the United States of America* 115:4027-4032 doi:10.1073/pnas.1700298114
16. Dai Z, Zhang X, Tang C, Muhammad N, Wu J, Brookes PC, Xu J (2017) Potential role of biochars in decreasing soil acidification-A critical review *Science of the Total Environment* 581:601-611
17. Demars BO, Edwards A (2007) Tissue nutrient concentrations in freshwater aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply *Freshwater Biology* 52:2073-2086
18. Dijkstra FA et al. (2016) Water, nitrogen and phosphorus use efficiencies of four tree species in response to variable water and nutrient supply *Plant Soil* 406:187-199
19. Du E et al. (2020) Global patterns of terrestrial nitrogen and phosphorus limitation *Nature Geoscience*:1-6
20. Du E, Vries WD, Han W, Liu X, Yuan J (2016) Imbalanced phosphorus and nitrogen deposition in China's forests *Atmospheric Chemistry & Physics* 16:8571-8579
21. Elser JJ et al. (2000) Biological Stoichiometry from Genes to Ecosystems *Ecology Letters* 3:540-550 doi:10.1111/j.1461-0248.2000.00185.x
22. Fang X-M et al. (2019) Phosphorus addition alters the response of soil organic carbon decomposition to nitrogen deposition in a subtropical forest *Soil Biology and Biochemistry* 133:119-128 doi:https://doi.org/10.1016/j.soilbio.2019.03.005
23. Fang Z, Shao C, Meng Y, Wu P, Chen M (2009) Phosphate signaling in *Arabidopsis* and *Oryza sativa* *Plant Science* 176:170-180
24. Fernández-Escobar R, Marin L, Sánchez-Zamora M, García-Novelo J, Molina-Soria C, Parra M (2009) Long-term effects of N fertilization on cropping and growth of olive trees and on N accumulation in soil profile *European Journal of Agronomy* 31:223-232
25. Ferretti M et al. (2015) On the tracks of Nitrogen deposition effects on temperate forests at their southern European range - an observational study from Italy *Global Change Biology* 20:3423-3438
26. Finzi AC, Austin A, Cleland EE, Frey S, Houlton BZ, Wallenstein MD (2011) Responses and feedbacks of coupled biogeochemical cycles to climate change: Examples from terrestrial ecosystems *Frontiers in Ecology and the Environment* 9:61-67 doi:10.1890/100001

27. Güsewell S (2004) N : P ratios in terrestrial plants: variation and functional significance *New Phytologist* 164:243-266 doi:10.1111/j.1469-8137.2004.01192.x
28. Güsewell S (2005) Nutrient resorption of wetland graminoids is related to the type of nutrient limitation *Functional Ecology* 19
29. Güsewell S, Koerselman W (2002) Variation in nitrogen and phosphorus concentrations of wetland plants *Perspectives in Plant Ecology, Evolution and Systematics* 5:37-61
30. Gilliam FS, Billmyer JH, Walter CA, Peterjohn WT (2016) Effects of excess nitrogen on biogeochemistry of a temperate hardwood forest: Evidence of nutrient redistribution by a forest understory species *Atmospheric Environment* 146:261-270 doi:https://doi.org/10.1016/j.atmosenv.2016.04.007
31. Gong Z et al. (2007) *Chinese Soil Taxonomy* BCAS
32. Greenwood DJ, Karpinets TV, Zhang K, Bosh-Serra A, Boldrini A, Karawulova L (2008) A unifying concept for the dependence of whole-crop N: P ratio on biomass: theory and experiment *Annals of botany* 102:967-977
33. Han W, Fang J, Guo D, Zhang Y (2005) Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China *The New phytologist* 168:377-385 doi:10.1111/j.1469-8137.2005.01530.x
34. Hessen DO, Ågren GI, Anderson TR, Elser JJ, De Ruiter PC (2004) Carbon sequestration in ecosystems: the role of stoichiometry *Ecology* 85:1179-1192
35. Huang J, Wang X, Yan E (2007) Leaf nutrient concentration, nutrient resorption and litter decomposition in an evergreen broad-leaved forest in eastern China *Forest Ecology and Management* 239:150-158 doi:https://doi.org/10.1016/j.foreco.2006.11.019
36. Huang Z, Liu B, Davis M, Sardans J, Peñuelas J, Billings S (2016) Long-term nitrogen deposition linked to reduced water use efficiency in forests with low phosphorus availability *New Phytologist* 210:431-442
37. Ingestad T (1974) Towards optimum fertilization *Ambio*:49-54
38. Killingbeck KT (1986) The terminological jungle revisited: making a case for use of the term resorption *Oikos* 46:263-264
39. Koerselman W, Meuleman AF (1996) The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation *Journal of applied Ecology*:1441-1450
40. Kou L, Wang H, Gao W, Chen W, Yang H, Li S (2016) Nitrogen addition regulates tradeoff between root capture and foliar resorption of nitrogen and phosphorus in a subtropical pine plantation *Trees* doi:10.1007/s00468-016-1457-7
41. Laliberté E, Turner BL, Costes T, Pearse SJ, Wyrwoll KH, Zemunik G, Lambers H (2012) Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot *Journal of Ecology* 100:631-642

42. Lebauer D, Treseder K (2008a) Nitrogen Limitation of Net Primary Productivity in Terrestrial Ecosystems is Globally Distributed *Ecology* 89:371-379 doi:10.1890/06-2057.1
43. LeBauer DS, Treseder KK (2008b) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed *Ecology* 89:371-379
44. Li Y, Niu S, Yu G (2016) Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: A meta-analysis *Global Change Biology* 22:934-943
45. Lin Y-M, Liu X-W, Zhang H, Fan H-Q, Lin G-H (2010) Nutrient conservation strategies of a mangrove species *Rhizophora stylosa* under nutrient limitation *Plant Soil* 326:469-479
46. Liu X et al. (2013) Enhanced nitrogen deposition over China *Nature* 494:459 doi:10.1038/nature11917
47. Macy P (1936) The quantitative mineral nutrient requirements of plants *Plant physiology* 11:749
48. McGroddy ME, Daufresne T, Hedin LO (2004) Scaling of C: N: P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios *Ecology* 85:2390-2401
49. Meunier CL, Gundale MJ, Sánchez IS, Liess A (2016) Impact of nitrogen deposition on forest and lake food webs in nitrogen-limited environments *Global Change Biology* 22:164-179
50. Mulligan D, Sands R (1988) Dry matter, phosphorus and nitrogen partitioning in three *Eucalyptus* species grown under a nutrient deficit *New phytologist* 109:21-28
51. Musick HB (1978) Phosphorus toxicity in seedlings of *Larrea divaricata* grown in solution culture *Botanical Gazette* 139:108-111
52. Ostertag R (2010) Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests *Plant Soil* 334:85-98
53. Pang D et al. (2019) Responses of soil labile organic carbon fractions and stocks to different vegetation restoration strategies in degraded karst ecosystems of southwest China *Ecological Engineering* 138:391-402 doi:https://doi.org/10.1016/j.ecoleng.2019.08.008
54. Penuelas J et al. (2013) Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe *Nature Communications* 4 doi:10.1038/ncomms3934
55. Phoenix GK et al. (2012) Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments *Global Change Biology* 18:1197-1215
56. Reich P, 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 doi:10.1073/pnas.0403588101
57. Rejmánková E (2005) Nutrient resorption in wetland macrophytes: comparison across several regions of different nutrient status *New Phytologist* 167:471-482 doi:10.1111/j.1469-8137.2005.01449.x
58. Sabaté S, Sala A, Gracia CA (1995) Nutrient content in *Quercus ilex* canopies: seasonal and spatial variation within a catchment *Plant Soil* 168:297-304

59. Sadanandan Nambiar EK, Fife DN (1991) Nutrient retranslocation in temperate conifers *Tree Physiol* 9:185-207 doi:10.1093/treephys/9.1-2.185
60. Sardans J et al. (2016a) Foliar and soil concentrations and stoichiometry of nitrogen and phosphorus across European *Pinus sylvestris* forests: relationships with climate, N deposition and tree growth *Functional Ecology* 30:676-689
61. Sardans J et al. (2016b) Foliar and soil concentrations and stoichiometry of nitrogen and phosphorus across European *Pinus sylvestris* forests: relationships with climate, N deposition and tree growth *Functional Ecology* 30:676-689
62. 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 doi:10.1890/15-0188.1
63. Shane M, Lambers H (2007) Phosphorus nutrition of Australian Proteaceae and Cyperaceae: a strategy on old landscapes with prolonged oceanically buffered climates *South African Journal of Botany* 73:274-275
64. Silla F, Escudero A (2003) Uptake, demand and internal cycling of nitrogen in saplings of Mediterranean *Quercus* species *Oecologia* 136:28-36 doi:10.1007/s00442-003-1232-5
65. Sistla SA, Schimel JP (2012) Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change *New Phytologist* 196:68-78
66. Standish R, Stokes B, Tibbett M, Hobbs R (2007) Seedling response to phosphate addition and inoculation with arbuscular mycorrhizas and the implications for old-field restoration in Western Australia *Environmental and Experimental Botany* 61:58-65
67. Sun Y, Hu R, Zhang C (2019) Does the adoption of complex fertilizers contribute to fertilizer overuse? Evidence from rice production in China *Journal of Cleaner Production* 219:677-685 doi:https://doi.org/10.1016/j.jclepro.2019.02.118
68. Tang X et al. (2018a) Carbon pools in China's terrestrial ecosystems: New estimates based on an intensive field survey *Proceedings of the National Academy of Sciences of the United States of America* 115:4021-4026 doi:10.1073/pnas.1700291115
69. Tang Z et al. (2018b) Patterns of plant carbon, nitrogen, and phosphorus concentration in relation to productivity in China's terrestrial ecosystems *Proceedings of the National Academy of Sciences* 115:4033-4038 doi:10.1073/pnas.1700295114
70. Tao L, Hunter MD (2012) Does anthropogenic nitrogen deposition induce phosphorus limitation in herbivorous insects? *Global Change Biology* 18:1843-1853
71. Tian D et al. (2019) Global changes alter plant multi-element stoichiometric coupling *New Phytologist* 221:807-817
72. Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants *Ecological Monographs* 82:205-220

73. Vitousek P, Porder S, Houlton B, Chadwick O (2010) Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions Ecological applications : a publication of the Ecological Society of America 20:5-15 doi:10.1890/08-0127.1
74. Wang M, Murphy MT, Moore TR (2014) Nutrient resorption of two evergreen shrubs in response to long-term fertilization in a bog Oecologia 174:365-377
75. Wright IJ, Westoby M (2003) Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species Functional Ecology 17:10-19
76. Wu TG, Yu MK, Wang GG, Dong Y, Cheng XR (2012) Leaf nitrogen and phosphorus stoichiometry across forty-two woody species in Southeast China Biochemical Systematics & Ecology 44:255–263
77. Xu S et al. (2017) Different spatial patterns of nitrogen and phosphorus resorption efficiencies in China's forests Scientific Reports 7:10584
78. Yan En-Rong, Wang Xi-Hua, Guo Ming, Zhong Qiang, Wu Z (2010) C:N:P stoichiometry across evergreen broad-leaved forests, evergreen coniferous forests and deciduous broad-leaved forests in the Tiantong region, Zhejiang Province, eastern China Chin J Plant Ecol 34:48-57 doi:10.3773/j.issn.1005-264x.2010.01.008
79. Yan Z, Li P, Chen Y, Han W, Fang J (2016) Nutrient allocation strategies of woody plants: an approach from the scaling of nitrogen and phosphorus between twig stems and leaves Scientific Reports 6 doi:10.1038/srep20099
80. Yang H (2018) Effects of nitrogen and phosphorus addition on leaf nutrient characteristics in a subtropical forest Trees 32:383-391
81. You C et al. (2018) Does foliar nutrient resorption regulate the coupled relationship between nitrogen and phosphorus in plant leaves in response to nitrogen deposition? Science of the Total Environment 645:733-742
82. Yu L, Zhu J, Mulder J, Dörsch P (2016) Multiyear dual nitrate isotope signatures suggest that N-saturated subtropical forested catchments can act as robust N sinks Global change biology 22:3662-3674
83. Yu Q et al. (2018) Threshold and multiple indicators for nitrogen saturation in subtropical forests Environmental Pollution 241:664-673
84. Yuan z (2015) Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes Nature Climate Change 5:465-469 doi:10.1038/nclimate2549
85. Yuan ZY, Chen HYH (2015) Negative effects of fertilization on plant nutrient resorption Ecology 96:373-380 doi:10.1890/14-0140.1
86. Zhang QF et al. (2017) Short-term effects of soil warming and nitrogen addition on the N:P stoichiometry of *Cunninghamia lanceolata* in subtropical regions Plant Soil 411:395-407 doi:10.1007/s11104-016-3037-4
87. Zhang R, Zhao Y, Lin J, Hu Y, Hänninen H, Wu J (2019) Biochar application alleviates unbalanced nutrient uptake caused by N deposition in *Torreya grandis* trees and seedlings Forest ecology and

88. Zhang Y, He X, Liang H, Zhao J, Zhang Y, Xu C, Shi X (2016) Long-term tobacco plantation induces soil acidification and soil base cation loss *Environmental Science and Pollution Research* 23:5442-5450
89. Zhao Q, Zeng D-H (2019) Nitrogen addition effects on tree growth and soil properties mediated by soil phosphorus availability and tree species identity *Forest Ecology and Management* 449:117478 doi:<https://doi.org/10.1016/j.foreco.2019.117478>
90. Zhu Z, Chen D (2002) Nitrogen fertilizer use in China—Contributions to food production, impacts on the environment and best management strategies *Nutrient Cycling in Agroecosystems* 63:117-127

Figures

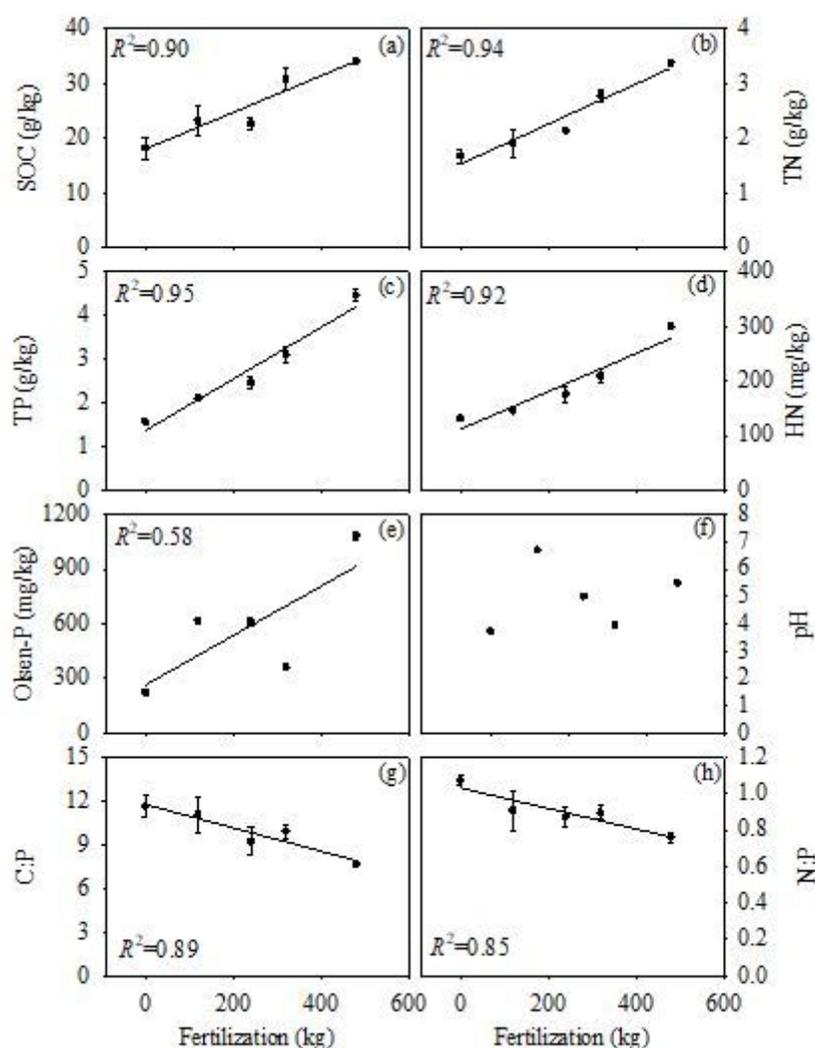


Figure 1

Relationship between nutrient supply and soil nutrient contents. SOC, TN, TP, HN, Olsen-P denote total soil organic C, Total N, Total P, Hydrolysable N, and Available P, respectively. Error bars refer to ± 1 standard

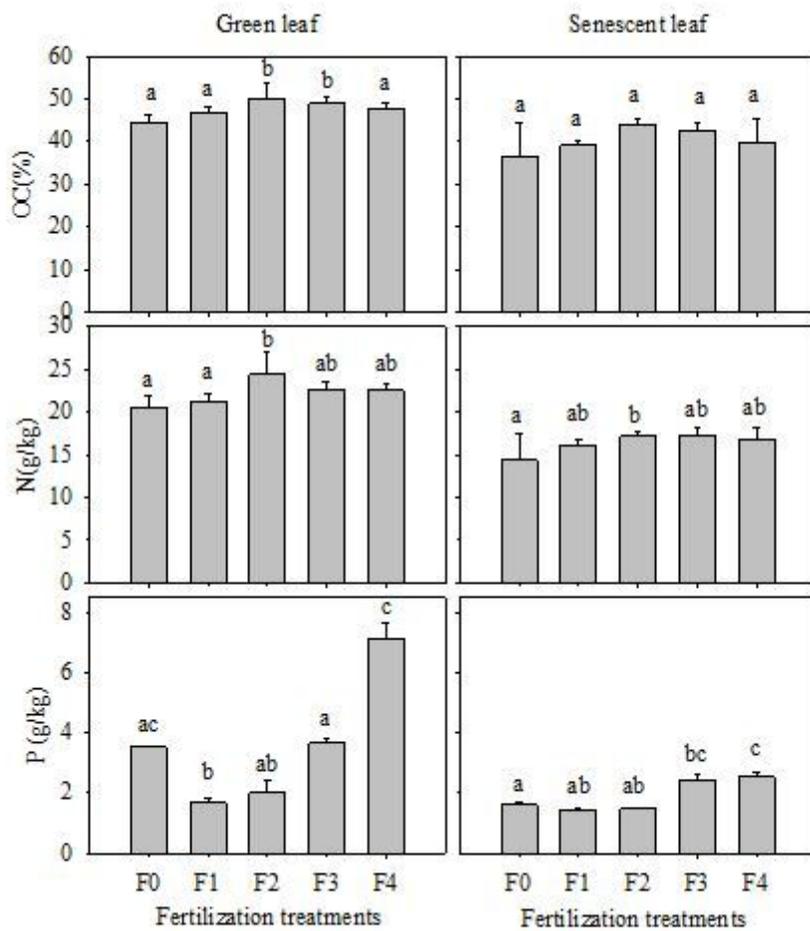


Figure 2

Effect of different fertilizer treatments on the leaves (left) and litters (right) nutrient contents (OC, N and P). Error bars refer to ± 1 standard error (a, b, c, d above each row indicate the differences in fertilizer treatments, $P < 0.05$.)

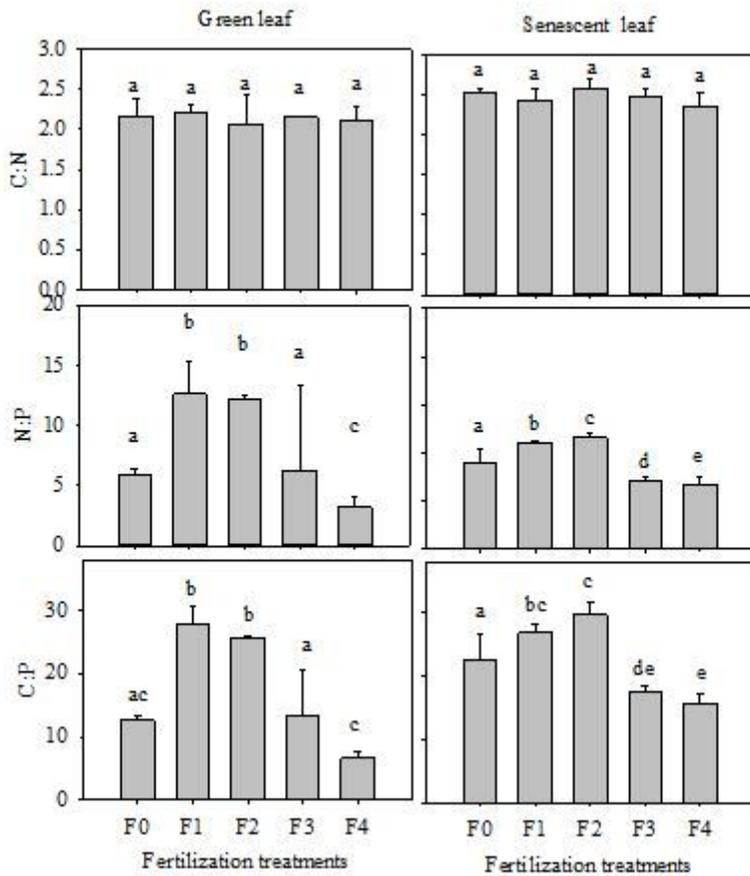


Figure 3

Green leaf stoichiometry under different fertilizer treatments. Error bars refer to ± 1 standard error (a, b above each row indicate the differences in fertilizer treatments, $P < 0.05$.)

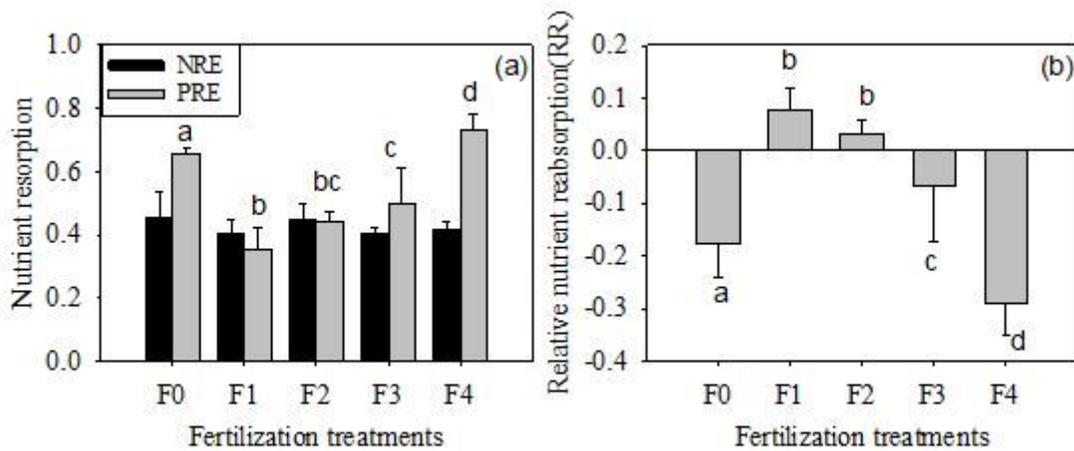


Figure 4

Nutrient resorption efficiency of N, P: a) NRE and PRE and b) relative nutrient resorption (RR) of *T. grandis* under different fertilizer treatments. Error bars above or below each row indicate the differences in

fertilizer treatments, $P < 0.05$.