Applying foliar stoichiometric traits of plants to determine fertilization for a mixed pine-oak stand in the Qinling Mountains, China

Lin Hou Corresponding Author, Zhenjie Dong, Yuanyuan Yang, Donghong Zhang, Shengli Zhang 1, Shuoxin Zhang 1

1 College of Forestry, Northwest A&F University, Yangling, Shaanxi, China
2 College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi, China

Corresponding Author: Lin Hou
Email address: houlin_1969@nwsuaf.edu.cn

Background. The Chinese Natural Forest Protection program has been conducted nationwide and has achieved resounding success. However, timber importation has increased; therefore, producing more domestic timber is critical to meet the demand for raw materials. Fertilization is one of the most effective silviculture practices used to improve tree and stand growth. However, determining the appropriate type and amount of elements is necessary for effective fertilization of big timber in different forest types and environmental conditions. Stoichiometric theory provides the criteria to assess nutrient limitation in plants and offers important insight into fertilizer requirements of forested ecosystems.

Methods. Nitrogen (N) and phosphorus (P) concentrations in plants’ leaves, mineral soil, and litter were investigated in a mixed pine-oak stand.

Results. The big timber rate for Pinus tabuliformis, Pinus armandii and Quercus aliena var. acutesserata is 57.71%, 22.79% and 2.78% of current existing individuals respectively. Foliar N and P concentrations were 9.08 and 0.88 mg g⁻¹, respectively. The N: P in the plants was 10.30. N concentration and N: P in mineral soil decreased from 0–30 cm soil depth. For litter, N and P concentrations were 16.89 and 1.51 mg g⁻¹, respectively, and N: P was 11.51. Concentrations of N and P in mineral soil and litter did not significantly affect plants’ leaf concentrations. Similar result was also obtained between litter and mineral soil concentrations. Nitrogen storage in mineral soil was significantly correlated with foliar N: P in the plants.

Discussion. Foliar N:P of dominant tree species and the plants, and foliar N concentration in Pinus tabuliformis and P. armandii, and foliar P concentration of P. armandii in the mixed pine-oak stand was lower than that in Chinese and other terrestrial plants. Foliar nutrients in the plants were not affected by soil nutrients. According to the criteria of nutrient limitation for plants, growth of dominant tree species was N limited; therefore, 1.49 t ha⁻¹ pure N should be added to forest land to as fertilizer.
Applying foliar stoichiometric traits of plants to determine fertilization for a mixed pine-oak stand in the Qinling Mountains, China

Lin Hou1*, Zhenjie Dong1, Yuanyuan Yang1, Donghong Zhang1, Shengli Zhang2, Shuoxin Zhang1

1 College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, China
2 College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, China

Corresponding author
Lin Hou

3 Taicheng Road, Yangling, Shaanxi, 712100, China
E-mail: houlin1969@163.com
Tel: +86-29-8708-2124
Abstract

Background. The Chinese Natural Forest Protection program has been conducted nationwide and has achieved resounding success. However, timber importation has increased; therefore, producing more domestic timber is critical to meet the demand for raw materials. Fertilization is one of the most effective silviculture practices used to improve tree and stand growth. However, determining the appropriate type and amount of elements is necessary for effective fertilization of big timber in different forest types and environmental conditions. Stoichiometric theory provides the criteria to assess nutrient limitation in plants and offers important insight into fertilizer requirements of forested ecosystems.

Methods. Nitrogen (N) and phosphorus (P) concentrations in plants’ leaves, mineral soil, and litter were investigated in a mixed pine-oak stand.

Results. The big timber rate for *Pinus tabuliformis*, *Pinus armandii* and *Quercus aliena* var. *acutisserata* is 57.71%, 22.79% and 2.78% of current existing individuals respectively. Foliar N and P concentrations were 9.08 and 0.88 mg g\(^{-1}\), respectively. The N: P in the plants was 10.30.

N concentration and N: P in mineral soil decreased from 0–30 cm soil depth. For litter, N and P concentrations were 16.89 and 1.51 mg g\(^{-1}\), respectively, and N: P was 11.51. Concentrations of N and P in mineral soil and litter did not significantly affect plants’ leaf concentrations. Similar result was also obtained between litter and mineral soil concentrations. Nitrogen storage in mineral soil was significantly correlated with foliar N: P in the plants.

Discussion. Foliar N:P of dominant tree species and the plants, and foliar N concentration in *Pinus tabuliformis* and *P. armandii*, and foliar P concentration of *P. armandii* in the mixed pine-
oak stand was lower than that in Chinese and other terrestrial plants. Foliar nutrients in the plants were not affected by soil nutrients. According to the criteria of nutrient limitation for plants, growth of dominant tree species was N limited; therefore, 1.49 t ha\(^{-1}\) pure N should be added to forest land to as fertilizer.
INTRODUCTION

The Natural Forest Protection Program (NFPP) was implemented in China in 1998 and has achieved or surpassed its initial goal by prohibiting commercial logging and partial or full harvesting of timber (Xu 2011). However, the effectiveness of the NFPP has been disputed as it has increased timber imports and caused deterioration in the structures of forest stands (Wang et al. 2013). To redress the insufficient production of domestic timber, the project “Techniques for big timber (diameter at breast height, DBH>26 cm) cultivation” has been conducted in both northern and southern forests in China. Besides proper forest management (Hou et al. 2017), applying fertilizer is also an efficient way to achieve “big timber”. However, determining the appropriate type and amounts of elements is necessary in the cultivation of big timbers in different forest types and environmental conditions.

Carbon (C), nitrogen (N), and phosphorus (P) are essential elements for plant growth and metabolic processes (Yang et al. 2015). The relative concentrations of C, N, and P in plants is known as stoichiometry (He et al. 2006), which is a unifying conceptual framework to examine how proportions of elements affect organisms and ecosystems (Danger et al. 2016). The most common limiting elements, C, N, and P, either individually or in combination, are widespread in terrestrial ecosystems (Venterink et al. 2001; Venterink et al. 2003; Vitousek et al. 2010).

Stoichiometric traits, particularly C: N and N: P, are useful indicators of nutrient limitation in both terrestrial ecosystems and ecosystem functioning (Elser et al. 2007; Gundersen et al. 2009).
In the last two decades, most research in ecological stoichiometry has focused on the causes and consequences of variation in C, N, and P ratios in organisms and their resources; however, there are large disparities in knowledge among taxa, ecosystem types, and specific research topics (Bell et al. 2014; Fanin et al. 2013; Han et al. 2005; He et al. 2006; Knoll et al. 2009; Sardans et al. 2011; Scharler et al. 2015; van Huysen et al. 2016; Venterink and Güsewell 2010; Vrede et al. 2004; Zhan et al. 2017). Leaf stoichiometry of plants, especially N and P, is very important in analyzing the composition, structure, and function of a community and ecosystem (Gao et al. 2013; Han et al. 2005; Rong et al. 2015; Venterink and Güsewell 2010). Determining how nutrients limit plant growth and N: P in leaves has become a hot topic (He et al. 2008).

Previous studies have documented N: P in plant leaves and biomass to infer or assess the degree of N or P limitation at the community level (Aerts and Chapin 1999; Ellison 2006; Güsewell 2004; Koerselman 1996; Sardans et al. 2012). However, determining the fertilizer of a specific community via ecological stoichiometry theory by assessing N and P limitation is still in its infancy (Finn et al. 2016; Hong et al. 2015). We hypothesized that growth of a dominant tree species would not be limited by nutrients when foliar N: P in the plants meet a threshold (Aerts and Chapin 1999; Güsewell 2004; Koerselman 1996; Sardans et al. 2012). We asked whether the amount of fertilization needed for a concrete forest stand could be determined using current N and P storage in mineral soil.

The Qinling Mountains are located in central China and have historically played a vital role in supplying timber for construction. The mixed pine-oak stand is extensive at the mid-altitudinal gradient of the Qinling Mountains (Hou et al. 2017). Maximizing the volume of this forest type
not only increases its carbon sequestration and ecosystem services, but also provides more timber for harvesting. The objectives of this study were to address key knowledge gaps, including: (1) foliar N and P stoichiometric traits in the plants in mixed pine-oak stand; (2) the correlation between N and P stoichiometry in forest soil and leaves in the plants; (3) nutrient limitation of the plants; and (4) preliminary recommendations for a fertilizer for dominant trees growing normally based on stoichiometric traits.

MATERIALS AND METHODS

Site description

Experiments were conducted at the Qinling National Forest Ecosystem Research Station (QNFERS), located on the southern slope of the Qinling Mountains, Huoditang, Ningshan County, Shaanxi Province, China (32°18′N, 108°20′E). The altitude of the study area was from 1500 to 2500 m. The area has subtropical climate, with annual mean air temperatures around 8–10°C, annual mean precipitation around 900–1200 mm, and annual mean evaporation around 800–950 mm. The main soil type was mountain brown soil, developed from granite material, ranging from 30–50 cm depth. The total forest area in the station was 2037 hectares. Natural forest occupied 93% of the total forest area in QNFERS, with various vegetation types distributed along an altitudinal gradient, such as evergreen deciduous mixed forest(mixed pine-oak forest), deciduous broad-leaved forest (oak, red birch), temperate coniferous forest (Chinese red pine, Armand pine), and cold temperate coniferous forest (spruce, fir). The most dominant forest type was mixed pine-oak forest restored after rotation felling between 1950 and 1998 with an average stand age of 42 years and an average height of 9.2 m. Common tree species included

*Pinus tabuliformis, P. armandii,* and *Quercus aliena* var. *acuteserrata,* associated with

*Toxicodendron vernicifluum, Carpinus turczaninowii, Swida macrophylla, Acer mono, Tilia*
paucicostata, Carpinus cordata, Juglans cathayensis, Bothrocaryum controversum, Rhus potaninii, Dendrobenhamia japonica var. Chinensis, Pterocarya stenoptera, Acer davidii, Betula albo-sinensis, Betula albo-sinensis var. septentrionalis, Juglans mandshurica, Rhus punjabensis Stewart var. sinica, Prunus padus, and Sorbus folgneri, and understory species were abundant (Hou et al. 2017).

Field investigations and sampling were conducted from 10 to 15 September, 2014. Samples of trees litter and soils were collected from 13 long-term fixed plots in the mixed pine-oak stand. Each plot was 20 ×20 m. Tree species, number, Height (H), and diameter at breast height (DBH) were recorded and used to determine the dominant tree species and DBH classes. Each increase in DBH by 4 cm is a DBH class and mid-diameter is used to stand for it (DBH ranging from 6.1 to 10 cm, the corresponding mid-diameter is 8 cm). One hundred and fifty-six of the average standard trees (AST) in all DBH classes were determined in each of the 13 plots (Supplementary). Mature, sunlit leaves without disease or insect pests were sampled. Four leaves/needles from each AST were collected in each of the four directions (north, east, south and west) and at different stem heights (crown, intermediate section, and underpart), and mixed into one sample for each AST. Five subplots (1 ×1 m) within each plot were randomly established to collect litter and mineral soil (depth 0–30 cm, 10 cm each layer). Samples of litter were collected manually and soil samples were collected by an auger (internal diameter 38 mm). Soil volumetric rings (100 cm³) were also used to collect soil samples to measure soil bulk density using the cutting ring method. Five samples of litter and mineral soil from the same depth within each subplot were mixed into one sample. All samples (litter and mineral soil) were weighed in the field before being transported to the laboratory.

Chemical analysis
Samples of leaves/needles and litter were oven-dried at 60 °C to a constant weight, and then ground using a plant sample mill and sieved through a 1-mm mesh screen. Nitrogen concentrations in leaves/needles and litter were measured using a flow injection analyzer (FIA5000, FOSS, Sweden), while P concentrations were measured using the molybdenum blue colorimetric method after digestion in a H_2SO_4+H_2O_2 solution (Bao 2000). Auger soil samples were air-dried under shade, then ground and sieved though a 0.149-mm mesh. Total soil N (TN) was measured using the Kjeldahl method (Kjeltec TM 8400, FOSS, Sweden), and total soil P (TP) was determined using acid digestion in a H_2SO_4+HClO_4 solution (Bao 2000).

**Data processing**

Importance values (IV) of tree species were calculated following Busby et al. (2010):

\[ RH = \frac{\sum H_i}{H} \times 100\% \]  
(1)

where, RH is the relative height, \( H_i \) is the height of tree species \( i \), and \( H \) is the height of all tree species in all plots.

\[ RF = \frac{\sum F_i}{F} \times 100\% \]  
(2)

where, RF is the relative frequency, \( F_i \) is the frequency of tree species \( i \), and \( F \) is the frequency of all tree species in all plots.

\[ RD = \frac{\pi \sum D_i^2}{D^2} \]  
(3)

where, RD is the relative basal area, \( D_i \) is the DBH of tree species \( i \), \( D \) is the basal area of all tree species in all plots, and \( f \) is the form factor of tree species (\( f_{\text{conifer}} = 0.40, f_{\text{broadleaf}} = 0.42 \)).

\[ IV = \frac{RH + RF + RD}{3} \times 100\% \]  
(4)

Tree species with IV>10% were determined as dominant. The big timber rate of dominant tree species was calculated as following.
\[ R_{bi} = \frac{N_{bi}}{N_i} \times 100\% \]

where \( R_{bi} \) is big timber rate (%) of tree species \( i \), \( N_{bi} \) is stems of big timber of tree species \( i \) and \( N_i \) is total stems of tree species \( i \) in the plots.

Nitrogen and P stoichiometry (N and P concentration and N: P) of plants’ leaves was calculated following Du et al. (2011):

\[ S_t = \sum_{i=1}^{n} C_{ni} \times IV_i \quad (5) \]

where, \( S_t \) is N and P stoichiometry of all tree species, \( C_{ni} \) is the concentration of N or P (mg \( g^{-1} \)) or N: P in leaves of tree \( i \), \( IV_i \) is the importance value of tree \( i \), and \( n \) is the number of dominant tree species.

Nutrient storage (t ha\(^{-1}\)) in litter was calculated as follows:

\[ S_l = C_l B_l \quad (6) \]

where, \( S_l \) is the litter nutrient storage (t ha\(^{-1}\)), \( C_l \) is element (N or P) concentration (mg \( g^{-1} \)), and \( B_l \) is the biomass (t ha\(^{-1}\)).

Storage of nutrients (N and P) in soil was calculated as follows:

\[ S_T = \sum_{i=1}^{n} E_i B_i D_i \quad (7) \]

where, \( S_T \) is the soil nutrient storage(tha\(^{-1}\)), \( E_i \) is the concentration of element \( i \) (mg \( g^{-1} \)), \( B_i \) is the bulk density (g cm\(^{-3}\)) in layer \( i \), and \( D_i \) is depth \( i \) (cm).

Differences of N, P concentrations and N: P between dominant tree species were analyzed using Statistical Analysis of the T-Test. The extremely significant difference (\( p<0.01 \)), significant difference (0.01<\( p<0.05 \)) and not significant difference (\( p>0.05 \)) between paired variables were demonstrated as different capital letters, different lowercase letters and the same lowercase letter respectively. The correlations between foliar N and P and N: P in the plants,
foliar N and P, N: P in the plants and mineral soil, and litter were assessed using Spearman’s rank correlation. Significance levels were set at $p=0.05$. All statistical analyses were performed using SPSS software (version 19.0 for Windows; SPSS Inc., Chicago, IL, USA). Figures were plotted using Origin 8.0 (OriginLab Corporation, Northampton, Massachusetts, USA).

RESULTS

Dominant tree species

There were 21 tree species in the mixed pine-oak stand. Aside from the photophilous species ($P. \text{tabuliformis}$, $P. \text{armandii}$, and $Q. \text{aliena var.acuteserrata}$), most of the species were shade-tolerant ($\text{Toxicodendron vernicifluum}$, $\text{Carpinus turczaninowii}$) or neutral with respective to light ($\text{Acer mono}$, $\text{Sorbus folgneri}$) (Table 1). The dominant tree species with importance values >10% were $P. \text{tabuliformis}$, $P. \text{armandii}$, and $Q. \text{aliena var.acuteserrata}$ (Table 1).

The big timber rate for $P. \text{tabuliformis}$, $P. \text{armandii}$, and $Q. \text{aliena var. acuteserrata}$ was 57.71%, 22.79% and 2.78% of current existing individuals respectively (Table 2).

Patterns of foliar N and P and N: P in the plants

Foliar N (11.84 ± 2.36 mg g$^{-1}$ to 21.72 ± 3.19 mg g$^{-1}$) and P (1.20 ± 0.14 mg g$^{-1}$ to 2.00 ± 0.31 mg g$^{-1}$) and N: P (9.62 to 10.87) exhibited large variation among tree species (Table 3). The general trend demonstrated that foliar nutrients and N: P of $P. \text{tabuliformis}$ and $P. \text{armandii}$ were less than that in $Q. \text{aliena var. acuteserrata}$ (Table 3). Moreover, foliar stoichiometric variables of the plants were also less than those in individual tree species in the mixed pine-oak stand (Table 3).
In the plants, concentrations of foliar N and P were significantly, positively correlated \((p=0.000)\) with each other (Figure 1(a)). The mathematical Log foliar N concentration was significantly \((p=0.02)\), positively correlated with the log N: P (Figure 1(b)). Foliar P concentration and N: P were significantly, negatively correlated \((p=0.000)\) with each other (Figure 1(c)).

**Patterns of N and P and N: P in mineral soil and litter**

Nitrogen concentrations and N: P varied markedly across mineral soil at 0–30 cm depth, ranging from 0.60±0.05 mg g\(^{-1}\) to 2.40±0.10 mg g\(^{-1}\) and 2.60±0.41 to 6.81±0.51, respectively (Table 4). However, P in mineral soil at 0–30 cm depth was significantly different, ranging from 0.23±0.03 mg g\(^{-1}\) to 0.36±0.03 mg g\(^{-1}\) (Table 4). Bulk density of mineral soil increased with soil depth (Table 4). The indices, mean N: P and concentrations of N and P in litter were 2.49, 12.06 and 5.21 times of which in mineral soil respectively (Table 4).

Correlations between N and P concentrations in litter and mineral soil were complex and none was significant (Figure 2(a) and (b)). At 0–10 cm depth, mineral soil N concentration increased with litter N concentration when litter N concentration was <17.9 mg g\(^{-1}\), and vice versa (Figure 2(a)). Soil P concentration increased with litter P concentration (Figure 2(b)). At 11–20 cm depth, mineral soil N concentration decreased with litter N concentration when litter N concentration was <19.0 mg g\(^{-1}\), and vice versa (Figure 2(a)). Soil P concentration showed an exponential correlation with litter P concentration (Figure 2(b)). At 21–30 cm depth, mineral soil N concentration increased with litter N concentration when litter N concentration was <17.9 mg.
g\(^{-1}\), and vice versa (Figure 2(a)). Phosphorus concentration of mineral soil increased linearly with litter P concentration (Figure 2(b)).

**Correlation between foliar N and P concentration in the plants and mineral soil**

Foliar N concentration in the plants increased with N concentration of mineral soil at 0-10 cm depth (Figure 3(a)). In contrast, foliar P concentration in the plants showed a significant, cubic correlation with P concentration of mineral soil (\(p=0.048\)) (Figure 3(b)). Although foliar N and P concentrations in the plants were not significantly (\(p=0.09\)) correlated with mineral soil at 11–20 cm depth, foliar N concentration in the plants increased with N concentration of mineral soil (Figure 3(a) and (b)). There was no correlation between foliar N concentration in the plants and N concentration of mineral soil at 21–30 cm depth (Figure 3(a)). Foliar P concentration in the plants increased with P concentration of mineral soil at 21–30 cm depth (Figure 3(b)).

**DISCUSSION**

**Foliar N and P stoichiometric traits in the plants in mixed pine-oak stand**

The positive correlation between foliar N and P concentrations (\(p=0.000\), Figure 1(a)) in the plants is consistent with stoichiometric stability criteria of a fixed ratio of nutrient absorption in the plant (Koerselman 1996). Previously, mean foliar N and P and N: P were reported as 18.6, 1.21 mg g\(^{-1}\), and 14.4, respectively for 753 species of terrestrial plants across China (Han et al. 2005) and 18.3, 1.42 mg g\(^{-1}\), and 11.8, respectively for 1251 world terrestrial plants (Reich and Oleksyn 2004). This study indicated that foliar N: P of dominant tree species (\(P.\) *tabuliformis*, \(P.\) *armandii*, and *Quercusaliena var. acuteserrata*) and in the plants (10.30:1) were lower than both Chinese and global terrestrial plants. Similarly, foliar N concentration of *P. tabuliformis*...
(11.84±1.40 mg g\(^{-1}\)) and \(P.\) \(armandii\) (12.98±1.43 mg g\(^{-1}\)), and foliar P concentration of \(P.\) \(armandii\) (1.20± 0.14 mg g\(^{-1}\)) were also lower. The overall element composition of plants in an ecosystem is determined by the mix of species and by the physiological status of the dominant plants (Güsewell 2004). Therefore, potential explanations for the observed patterns are that foliar element concentrations and ratios were strongly determined by genetic and physiological controls, and that these crucial factors prevented plants from responding to the natural availability of nutrients (Castle and Neff 2009). Furthermore, the nutrient status of terrestrial plants has a strong local and regional signal due to acquiring nutrients via weathering and microbial decomposition \textit{in situ} (Chadwick et al. 1999). In the study area, low air temperature (<10°C) (Hou et al. 2017) may have hindered rock weathering and microbial activity. However, the concentration of foliar N (21.72± 3.19 mg g\(^{-1}\)) and P (2.00± 0.31 mg g\(^{-1}\)) in \(Q.\) \(aliena\) var. \textit{acuteserrata} was higher than that reported for Chinese and global mean levels. Foliar P concentration of \(P.\) \textit{tabuliformis} (1.23± 0.22 mg g\(^{-1}\)) was higher than the Chinese mean, but lower than the global mean. The growing period (from mid-May to late-September) in the study area was relatively short for plots on high elevation (more than 1600 m) (Hou et al. 2017). The leaf life span of \(P.\) \textit{tabuliformis} and \(P.\) \textit{armandii} in the study area is 3 years. In the contrary, the leaf life span of \(Q.\) \textit{aliena} var. \textit{acuteserrata} is only 5 months. Therefore, shorter leaf life span and growing season may have caused higher N and P concentrations (Castle and Neff 2009; He et al. 2006).

**Response of foliar nutrients in the plants to soil nutrients**
The N and P concentrations in mineral soil were generally low and the similar result was also found by Wu (2015). The N concentration in the top layer of mineral soil (0–10 cm) was above the standard of first class soil (>2.0 g kg\(^{-1}\)) (National Soil Survey Office of China 1998); however, at 11–20 cm depth it only met the standard of third class soil (1–1.5 g kg\(^{-1}\)), and at 21–30 cm it met the standard of fifth class soil (0.5–0.75 g kg\(^{-1}\)) (National Soil Survey Office of China 1998). The level of soil P concentration at 0–30 cm only reached the standard of third class soil (0.2–0.4 g kg\(^{-1}\)) (National Soil Survey Office of China 1998). Litter decomposition and rock weathering are the main nutrient sources for mineral soil in natural forests. Plant element concentrations are largely determined by supplies of elements in soil and the chemical and physical characteristics of soil environments (Castle and Neff 2009). However, our findings demonstrated that elements in litter did not significantly affect mineral soil. We found most of the correlations between foliar N and P concentrations in the plants and mineral soil at various depths were not significant, and soil nutrients did not explain more than 14% of foliar nutrients in the plants. Therefore, these results were not able to provide a better understanding and interpretation of the effects of litter and soil nutrients on mineral soil and foliar nutrients in the plants. Possible explanations for the observed patterns include high non-soluble chemical compounds in litter and low temperature (Hou et al. 2017), which slowed down litter decomposition. Furthermore, soil available N and P absorbed by plants have strong mobility and are easily leached (Thompson et al. 2010). The thickness of mineral soil (at 30–50 cm depth), pore ratio from 32.0 to 62.28%, high precipitation (800–1200 mm), and low temperature (Hou et al. 2010, 2017) in the study area may have accelerated leaching of N and P in decomposed litter and mineral soil, leaving less N and P to be assimilated into leaves of the plants. In addition,
forest harvesting in 1950-1998 was also a key cause to decrease N and P concentrations in mineral soil.

**Nutrient limitation of plants**

It has been suggested that biomass N: P may be a better indicator of N or P deficiency than nutrient concentrations (Güsewell et al. 2003). Previous studies have reported that plant growth is limited by concurrent N concentration <20 mg g\(^{-1}\) and N: P<14; while P limits plant growth at concentrations of<1 mg g\(^{-1}\) and N: P >16. Therefore, co-limitation of N and P will occur when the concentrations and ratio of N and P meet these conditions (Aerts and Chapin 1999; Koerselman 1996). The foliar N: P (10.3) and N concentration (9.08 mg g\(^{-1}\)) of the plants in the mixed pine-oak (Table 3) stand showed N limitation according to above mentioned criteria (Aerts and Chapin 1999; Koerselman 1996).

The correlations between soil nutrient storage and foliar N: P of the plants indicated that foliar N: P of the plants was correlated with soil N storage (Figure 4(a)). One possible reason may be that the growth of the plants was limited by N, and foliar N: P of the plants responses to soil N were more sensitive. However, N storage in mineral soil only explained 40% (Figure 4(a)) of the variation in foliar N: P of the plants, and foliar N: P of the plants was not significantly correlated with soil P storage (Figure 4(b)). This may have been a result of low P concentration in mineral soil, which also affected foliar N: P of the plants.

Our results showed that the total standing crop of elements in litter was estimated as 0.31±0.07 t ha\(^{-1}\) for N, and 0.02±0.01 t ha\(^{-1}\) for P; and 4.58±0.15 t ha\(^{-1}\) for N and 0.95±0.09 t ha\(^{-1}\) for P in mineral soil at 0–30 cm depth (Table 4). This suggested that trees might assimilate N and P entirely from that stored in mineral soil and litter. According to the criteria (Aerts and Chapin...
1999; Koerselman 1996), 1.49 t ha$^{-1}$ pure N should be used to fertilize forest land to relieve N limitation on tree growth.

**Limitations of the analysis**

We analyzed stoichiometric traits of leaves in the plants, mineral soil, and litter to explore the effects of N and P concentrations and N: P of litter and mineral soil on leaves in the plants. We also investigated the correlations between foliar N: P in the plants and N and P storage in mineral soil. The factors included in this study only explained 40% of the variation, which prevented us from fully understanding and interpreting total variation. Furthermore, besides criteria we used in the study, a more conservative estimate of N: P threshold is <10 for N limitation and >20 for P limitation (Güsewell 2004; Sardans et al. 2012). Since there are multiple criteria for assessing nutrient limitation (Aerts and Chapin 1999; Güsewell 2004; Koerselman 1996; Sardans et al. 2012), we lack sufficient evidence to support an accurate estimation of fertilizer application rate. Finally, nutrient use efficiency and its influence on tree growth was not included in the estimation of pure N application rate, as it would have underestimated N. To verify these preliminary results, more study is required to detect the effects of fertilizer on stoichiometric traits of trees and mineral soil, and N and P interactions.

**CONCLUSIONS**

Our results indicated that N and P concentrations were low in leaves in the plants and mineral soil, but high in litter. Concentrations of N and P in mineral soil were insufficient to explain the variation in foliar N: P in the plants. However, N storage in mineral soil at 0–30 cm depth was correlated with foliar N: P. The growth of the plants was limited by N; therefore, the stoichiometric approach we used in the present study reveals that approximately 1.49 t ha$^{-1}$ of N should be added if we want to achieve big timber cultivation. However, these rates should be
further confirmed by a field fertilization experiments. We further recommend that appropriate fertilization rates should be included in the process of big timber cultivation. This result offers important insights into fertilizing forest land.

**ACKNOWLEDGEMENTS**

We thank staff at Huoditang Forest Farm, Northwest A&F University for their assistance with sampling.

**Data Availability**

The following information was supplied regarding data availability:

The raw data has been supplied as Supplementary File.
REFERENCES

Aerts R, and Chapin FSI. 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research **30**:1-67.

Bao SD. 2000. Soil and Agricultural Chemistry Analysis. Beijing: China Agriculture Press.

Bell C, Carrillo Y, Boot CM, Rocca JD, Pendent E, and Wallenstein MD. 2014. Rhizosphere stoichiometry: are C : N : P ratios of plants, soils, and enzymes conserved at the plant species-level? *New Phytologist* **201**:505-517.

Busby PE, Vitousek P, and Dirzo R. 2010. Prevalence of tree regeneration by sprouting and seeding along a rainfall gradient in Hawai‘i. *Biotropica* **42**:80-86.

Castle SC, and Neff JC. 2009. Plant response to nutrient availability across variable bedrock geologies. *Ecosystems* **12**:101-113.

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, and Hedin LO. 1999. Changing sources of nutrients during four million years of ecosystem development. *Nature* **397**:491-497.

Danger M, Gessner MO, and Bärlocher F. 2016. Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. *Fungal Ecology* **19**:100-111.

Du Y, Pan G, Li L, Hu Z, and Wang X. 2011. Leaf N/P ratio and nutrient reuse between dominant species and stands: predicting phosphorus deficiencies in Karst ecosystems, southwestern China. *Environmental Earth Sciences* **64**:299-309.

Ellison AM. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology* **8**:740-747.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, and Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**:1135-1142.

Fanin N, Fromin N, Buatois B, and Hättenschwiler S. 2013. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter–microbe system.
Finn D, Page K, Catton K, Kienzle M, Robertson F, Armstrong R, and Dalal R. 2016. Ecological stoichiometry controls the transformation and retention of plant-derived organic matter to humus in response to nitrogen fertilisation. *Soil Biology and Biochemistry* 99:117-127.

Güsewell S. 2004. N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist* 164:243-266.

Güsewell S, Koerselman W, and Verhoeven JTA. 2003. Biomass N: P ratios as indicators of nutrient limitation for plant populations in wetlands. *Ecological Applications* 13:372-384.

Gao Y, Yu G, and He N. 2013. Equilibration of the terrestrial water, nitrogen, and carbon cycles: advocating a health threshold for carbon storage. *Ecological Engineering* 57:366-374.

Gundersen P, Sevel L, Christiansen JR, Vesterdal L, Hansen K, and Bastrup-Birk A. 2009. Do indicators of nitrogen retention and leaching differ between coniferous and broadleaved forests in Denmark? *Forest Ecology and Management* 258:1137-1146.

Han W, Fang J, Guo D, and Zhang Y. 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytologist* 168:377-385.

He JS, Fang J, Wang Z, Guo D, Flynn DFB, and Geng Z. 2006. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China. *Oecologia* 149:115-122.

He JS, Wang L, Dan FBF, Wang X, Ma W, and Fang J. 2008. Leaf Nitrogen:Phosphorus stoichiometry across Chinese grassland biomes. *Oecologia* 155:301-310.

Hong J, Wang X, and Wu J. 2015. Effects of soil fertility on the N:P stoichiometry of herbaceous plants on a nutrient-limited alpine steppe on the northern Tibetan Plateau. *Plant and Soil* 391:179-194.

Hou L, Lei R, Zhang S, and Liu J. 2010. Temporal and spatial variations of soil respiration in *Pinus tabulaeformis* forest at Huoditang forest zone in the Qinling Mountains, China.
Acata Ecologica Sinica 30:5225-5236.

Hou L, Sun S, Liang L, Liang G, and Jiang L. 2017. Effects of selective thinning and residue removal on ground layer structure and diversity in a mixed pine–oak stand of the Qinling Mountains, China. Australian Journal of Botany 65:485-496.

Knoll LB, McIntyre PB, Vanni MJ, and Flecker AS. 2009. Feedbacks of consumer nutrient recycling on producer biomass and stoichiometry: separating direct and indirect effects. Oikos 118:1732-1742.

Koerselman W. 1996. The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. Journal of Applied Ecology 33:1441-1450.

National Soil Survey Office of China. 1998. Soils of China. Beijing, China: Chinese Agriculture Press.

Reich PB, and 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.

Rong Q, Liu J, Cai Y, Lu Z, Zhao Z, Yue W, and Xia J. 2015. Leaf carbon, nitrogen and phosphorus stoichiometry of Tamarix chinensis Lour. in the Laizhou Bay coastal wetland, China. Ecological Engineering 76:57-65.

Sardans J, Rivas-Ubach A, and Peñuelas J. 2011. Factors affecting nutrient concentration and stoichiometry of forest trees in Catalonia (NE Spain). Forest Ecology and Management 262:2024-2034.

Sardans J, Rivasubach A, and Peñuelas J. 2012. The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives. Biogeochemistry 111:1-39.

Scharler UM, Ulanowicz RE, Fogel ML, Wooller MJ, Jacobson-Meyers ME, Lovelock CE, Feller IC, Frischer M, Lee R, McKee K, Romero I C, Schmit J P, Shearer C. 2015. Variable nutrient stoichiometry (carbon:nitrogen:phosphorus) across trophic levels
determines community and ecosystem properties in an oligotrophic mangrove system. 

*Oecologia* **179:**863-876.

**Thompson K, Parkinson JA, Band SR, and Spencer RE. 2010.** A comparative study of leaf nutrient concentrations in a regional herbaceous flora. *New Phytologist* **136:**679-689.

**van Huysen TL, Perakis SS, and Harmon ME. 2016.** Decomposition drives convergence of forest litter nutrient stoichiometry following phosphorus addition. *Plant and Soil* **406:**1-14.

**Venterink HO, and Güsewell S. 2010.** Competitive interactions between two meadow grasses under nitrogen and phosphorus limitation. *Functional Ecology* **24:**877-886.

**Venterink HO, Vliet REVD, and Wassen MJ. 2001.** Nutrient limitation along a productivity gradient in wet meadows. *Plant and Soil* **234:**171-179.

**Venterink HO, Wassen MJ, Verkroost AWM, and Ruiter PCD. 2003.** Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. *Ecology* **84:**2191-2199.

**Vitousek PM, Porder S, Houlton BZ, and Chadwick OA. 2010.** Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications A Publication of the Ecological Society of America* **20:**5.

**Vrede T, Dobberfuhl DR, Kooijman SALM, and Elser JJ. 2004.** Fundamental connections among organism C:N:P stoichiometry, macromolecular composition, and growth. *Ecology* **85:**1217-1229.

**Wang W, Pechacek P, Zhang M, Xiao N, Zhu J, and Li J. 2013.** Effectiveness of nature reserve system for conserving tropical forests: a statistical evaluation of Hainan Island, China. *Plos One* **8:**e57561.

**Wu H. 2015.** The relationship between terrain factors and spatial variability of soil nutrients for pine-oak mixed forest in Qinling Mountains. *Journal of Natural Resources* **30:** 858-869.

**Xu J. 2011.** China's new forests aren't as green as they seem. *Nature* **477:**371-371.
Yang X, Huang Z, Zhang K, and Cornelissen JHC. 2015. C:N:P stoichiometry of Artemisia species and close relatives across northern China: unravelling effects of climate, soil and taxonomy. *Journal of Ecology* **103**:1020-1031.

Zhan S, Wang Y, Zhu Z, Li W, and Bai Y. 2017. Nitrogen enrichment alters plant N: P stoichiometry and intensifies phosphorus limitation in a steppe ecosystem. *Environmental and Experimental Botany* **134**:21-32.
Table 1 (on next page)

Importance value of tree species

RH, RD, RF and IV in the table represent relative frequency, relative basal area and important value of tree species respectively.
Table 1: Importance value of tree species

| Species                          | RH (%) | RD (%) | RF (%) | IV (%) |
|----------------------------------|--------|--------|--------|--------|
| Pinus tabuliformis               | 30.9   | 65.2   | 9.4    | 35.2   |
| Pinus armandi                    | 24.1   | 27.5   | 8.7    | 20.1   |
| Quercus aliena var. acuteserrata | 16.3   | 5.1    | 10.2   | 10.6   |
| Toxicodendron vernicifluum       | 7.0    | 1.3    | 8.7    | 5.6    |
| Carpinus turczaninowii           | 2.2    | 0.1    | 8.7    | 3.6    |
| Swida macrophylla                | 5.0    | 0.4    | 5.5    | 3.6    |
| Acer mono                        | 2.0    | 0.1    | 7.9    | 3.3    |
| Tilia paucicostata               | 1.6    | 0.1    | 6.3    | 2.6    |
| Carpinus cordata                 | 1.3    | 0.0    | 5.5    | 2.3    |
| Juglans cathayensis              | 1.3    | 0.1    | 4.7    | 2.0    |
| Bothrocaryum controversum        | 2.2    | 0.1    | 3.1    | 1.8    |
| Rhus potaninii                   | 0.7    | 0.0    | 4.7    | 1.8    |
| Dendrobatanция japonica var. chinensis | 0.7  | 0.0    | 3.9    | 1.6    |
| Pterocarya stenoptera            | 2.2    | 0.1    | 2.4    | 1.5    |
| Acer davidii                     | 1.1    | 0.0    | 3.1    | 1.4    |
| Betula albo-sinensis             | 0.9    | 0.0    | 3.1    | 1.3    |
| Betula albo-sinensis var. septentrionalis | 0.2  | 0.0    | 0.8    | 0.3    |
| Juglans mandshurica              | 0.2    | 0.0    | 0.8    | 0.3    |
| Rhus punjabensis Stewart var. sinica | 0.1  | 0.0    | 0.8    | 0.3    |
| Prunus padus                     | 0.1    | 0.0    | 0.8    | 0.3    |
| Sorbus folgneri                  | 0.1    | 0.0    | 0.8    | 0.3    |
| **Sum**                          | **100.0** | **100.0** | **100.0** | **100.0** |

Note: RH, RD, RF and IV in the table represent relative frequency, relative basal area and important value of tree species respectively.
Table 2 (on next page)

DBH distribution of dominant tree species

Big timber rate of dominant tree species was calculated in text.
| Mid-diameter (cm) | Pinus tabuliformis | Pinus armandi | Quercus aliena var. acuteserrata |
|-------------------|--------------------|---------------|---------------------------------|
| 4                 | 0.65               | 1.47          | 38.89                           |
| 8                 | 2.58               | 3.68          | 38.89                           |
| 12                | 3.23               | 18.38         | 10.19                           |
| 16                | 5.81               | 20.59         | 3.70                            |
| 20                | 16.77              | 18.38         | 3.70                            |
| 24                | 12.26              | 14.71         | 1.85                            |
| 28                | 23.87              | 8.82          | 2.78                            |
| 32                | 14.19              | 5.88          |                                 |
| 36                | 10.32              | 5.15          |                                 |
| 40                | 7.10               | 2.94          |                                 |
| 44                | 2.58               |               |                                 |
| 48                | 0.65               |               |                                 |
| sum               | 100                | 100           | 100                             |
Table 3 (on next page)

Leaf stoichiometric traits of plants

TN, TP, N: P and IV in the table represents leaf concentration of total nitrogen, phosphorus and N: P ratio both in dominant tree species and plants, and importance value of tree species respectively. Extremely significant differences ($p=0.000$) of leaf TN were between tree species. Extremely significant differences ($p=0.000$) of leaf TP were between *Quercus aliena* var. *acuteserrata*-*Pinus tabuliformis* and *Quercus aliena* var. *acuteserrata*-*Pinus armandi*. Not significant difference ($p=0.88$) of leaf TP was between *Pinus tabuliformis*-*Pinus armandi*. Significant differences of leaf N:P were between *Pinus tabuliformis*-*Pinus armandi* ($p=0.048$) and *Pinus tabuliformis*-*Quercus aliena* var. *acuteserrata* ($p=0.04$). Not significant difference ($p=0.91$) of leaf N:P was between *Quercus aliena* var. *acuteserrata*-*Pinus armandi*. 
Table 3 Leaf stoichiometric traits of plants

| Tree species                  | TN (mg·g⁻¹) | TP (mg·g⁻¹) | N:P       | IV (%)  | Plants                       |
|-------------------------------|-------------|-------------|-----------|---------|-----------------------------|
| Pinus tabuliformis            | 11.84±1.40 A| 1.23±0.22 a | 9.62:1 a  | 35.2    |                             |
| Pinus armandi                 | 12.98±1.43 B| 1.20±0.14 a | 10.82:1 b | 20.1    | 9.08 0.88 10.30:1           |
| Quercus aliena var. acuteserrata | 21.72±3.19 C| 2.00±0.31 A | 10.87:1 b | 10.6    |                             |

Note: TN, TP, N: P and IV in the table represents leaf concentration of total nitrogen, phosphorus and N: P ratio both in dominant tree species and plants, and importance value of tree species respectively. Extremely significant differences (p=0.000) of leaf TN were between tree species. Extremely significant differences (p=0.000) of leaf TP were between Quercus aliena var. acuteserrata-Pinus tabuliformis and Quercus aliena var. acuteserrata-Pinus armandi. Not significant difference (p=0.88) of leaf TP was between Pinus tabuliformis- Pinus armandi. Significant differences of leaf N:P were between Pinus tabuliformis- Pinus armandi (p=0.048) and Pinus tabuliformis- Quercus aliena var. acuteserrata (p=0.04). Not significant difference (p=0.91) of leaf N:P was between Quercus aliena var. acuteserrata-Pinus armandi.
Leaf stoichiometric traits of plants

TN, TP, N: P and IV in the table represents leaf concentration of total nitrogen, phosphorus and N: P ratio both in dominant tree species and plants, and importance value of tree species respectively. Extremely significant differences (p=0.000) of leaf TN were between tree species. Extremely significant differences (p=0.000) of leaf TP were between Quercus aliena var. acuteserrata-Pinus tabuliformis and Quercus aliena var. acuteserrata-Pinus armandii. Not significant difference (p=0.88) of leaf TP was between Pinus tabuliformis-Pinus armandi. Significant differences of leaf N:P were between Pinus tabuliformis- Pinus armandi (p=0.048) and Pinus tabuliformis- Quercus aliena var. acuteserrata (p=0.04). Not significant difference (p=0.91) of leaf N:P was between Quercus aliena var. acuteserrata-Pinus armandi.
Table 4. Stoichiometric traits of mineral soil and litter

| Component | Depth (cm) | TN (mg g⁻¹) | TP (mg g⁻¹) | N:P ratio | Bulk density (g cm⁻³) | Biomass (t ha⁻¹) | Nutrients storage (t ha⁻¹) |
|-----------|-----------|-------------|-------------|-----------|-----------------------|-----------------|---------------------------|
|           |           |             |             |           |                       |                 |                           |
|           | 0-10      | 2.40±0.10   | 0.36±0.03   | (6.81:1)  | 0.51                  | 0.10±0.02       |                           |
| Mineral   | 11-20     | 1.20±0.04   | 0.27±0.03   | (4.45:1)  | 0.39                  | 1.15±0.05       | 4.58±0.15                 |
| soil      | 21-30     | 0.60±0.05   | 0.23±0.03   | (2.60:1)  | 0.41                  | 1.22±0.06       | 0.95±0.09                 |
| Mean      |           | 1.40        | 0.29        | 4.62      |                       |                 |                           |
| Litter    |           | 16.89±3.59  | 1.51±0.24   | (11.51:1) | 3.20                  | 18.66±1.78      | 0.31±0.07                 |

Note: TN, TP and N: P in the table represents leaf concentration of total nitrogen, phosphorus and N: P ratio both in soil and litter respectively.
Figure 1

Interaction of foliar N, P concentrations and N: P ratio of plants.

(a) Leaf concentrations of N and P in plants. (b) Leaf concentration of N and N:P in plants. (c) Leaf concentration of P and N:P in plants.
Figure 2

Interaction of litter nutrients and mineral soil nutrients.

- Nitrogen concentrations of litter and miner soil within 0-10 cm.
- Nitrogen concentrations of litter and miner soil within 11-20 cm.
- Nitrogen concentrations of litter and miner soil within 21-30 cm.
- Phosphorus concentrations of litter and miner soil within 0-10 cm.
- Phosphorus concentrations of litter and miner soil within 11-20 cm.
- Phosphorus concentrations of litter and miner soil within 21-30 cm.

(a) Nitrogen concentrations of litter and mineral soil within 0-10 cm.
(b) Phosphorus concentrations of litter and mineral soil within 0-10 cm.
Figure 3

Mineral soil nutrients and foliar nutrients of plants interaction.

- Nitrogen concentrations of miner soil within 0-10 cm and leaf in plants.
- Nitrogen concentrations of miner soil within 11-20 cm and leaf in plants.
- Nitrogen concentrations of miner soil within 21-30 cm and leaf in plants.
- Phosphorus concentrations of miner soil within 0-10 cm and leaf in plants.
- Phosphorus concentrations of miner soil within 11-20 cm and leaf in plants.
- Phosphorus concentrations of miner soil within 21-30 cm and leaf in plants.
Figure 4

Relationships between storage of soil nutrients and foliar N: P ratio of plants.

(a) Relationship between nitrogen storage of mineral soil and N: P ratio of plants. (b) Relationship between phosphorus storage of mineral soil and N: P ratio of plants.