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

Lin Hou[1]*, Zhenjie Dong[1], Yuanyuan Yang[1], Donghong Zhang [1],Shengli Zhang[2], Shuoxin Zhang[1]

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 fertilizing in forest land.

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

Results. The big timber rate of *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 mgg^{-1}, respectively. The N: P in the tree layer 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 $mgg^{-1,}$ respectively, and N: P was 11.51. Concentrations of N and P in mineral soil and litter did not significantly affect tree layer leaves or mineral soil. Nitrogen storage in mineral soil was significantly correlated with foliar N: P in the tree layer.

Discussion. Foliar N:P of dominant tree species and the tree layer, and foliar N concentration in *Pinus tabuliformis* and *P. armandii*, and foliar P concentration of *P. armandii* in the mixed pine-

Commented [AOA1]: fertilizer requirements of forested ecosystems

Commented [AOA2]: What does this mean? Is it the rate at which big timber of each of the three listed species is found in the stand studied? Please rephrase the sentence so its meaning is clearly comprehensible. See Line 192

Commented [AOA3]: 10.32

Commented [AOA4]: 11.19

Commented [AOA5]: ……tree layer leaf concentrations. Similar result was also obtained between litter and mineral soil concentrations.

oak stand was lower than that in Chinese and other terrestrial plants. Foliar nutrients in the tree layer 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 was added to forest land to as fertilizer.

Keywords: nitrogen; phosphorus; N: P; fertilizer; mixed pine-oak stand; the Qinling Mountains

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 these elements affect organisms and ecosystems,. with element limitation one of the most important topics (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

Formatted: Font color: Red

Commented [AOA6]: What exactly does dangler et al 2016 say? The phrase "……with element limitation one of the most important topics (Danger at al. 2016)" is hard to understand. Consider a period after "…. These elements affect organisms and ecosystems."

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 tree layer meets 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 tree layer in mixed pine-oak stand; (2) the relationship between N and P stoichiometry in forest soil and leaves in the tree layer; (3) nutrient limitation of the tree layer; 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 ~~experienced a~~ 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 with an average stand age of 42 years and an average height of 9.2 m. Common tree species included *Pinus tabuliformis*, *P.*

Formatted: Font color: Red

Formatted: Font color: Red

126 *armandii*, and *Quercusaliena* var. *acuteserrata*, and understory species were abundant (Hou et
127 al. 2017).
128 Field investigations and sampling were conducted from 10 to 15 September, 2014. Samples
129 of trees litter and soils were collected from 13 long-term fixed plots in the mixed pine-oak stand.
130 Each plot was 20 ×20 m. Tree species, number, Height (H), and diameter at breast height (DBH)
131 were recorded and used to determine the dominant tree species and DBH classes. DBH
132 increasing 4 cm each is a DBH class and mid-diameter is used to stand for it (DBH ranging from
133 6.1 to 10 cm, the corresponding mid-diameter is 8 cm). One hundred and fifty-six of the average
134 standard trees (AST) in all DBH classes were determined in each of the 13 plots
135 (Supplementary). Mature, sunlit leaves without disease or insect pests were sampled. Four
136 leaves/needles from each AST were collected in each of the four directions (north, east, south
137 and west) and at different stem heights (crown, intermediate section, and underpart), and mixed
138 into one sample for each AST. Five subplots (1 ×1 m) within each plot were randomly
139 established to collect litter and mineral soil (depth 0–30 cm, 10 cm each layer). Samples of litter
140 were collected manually and soil samples were collected by an auger (internal diameter 38 mm).
141 Soil volumetric rings (100 cm^3) were also used to collect soil samples to measure soil bulk
142 density using the cutting ring method. Five samples of litter and mineral soil from the same
143 depth within each subplot were mixed into one sample. All samples (litter and mineral soil)
144 were weighed in the field before being transported to the laboratory.
145 **Chemical analysis**
146 Samples of leaves/needles and litter were oven-dried at 60 °C to a constant weight, and
147 then ground using a plant sample mill and sieved through a 1-mm mesh screen. Nitrogen
148 concentrations in leaves/needles and litter were measured using a flow injection analyzer
149 (FIA5000, FOSS, Sweden), while P concentrations were measured using the molybdenum blue

Commented [AOA7]: Rephrase this sentence to show the BDH classes used in the study. The sentence in its present form is hard to understand.

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):

$$RH = \frac{\sum H_i}{H} \times 100\% \quad (1)$$

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.

$$RF = \frac{\sum F_i}{F} \times 100\% \quad (2)$$

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.

$$RD = \frac{\frac{1}{4}\pi f \sum D_i^2}{D^2} \quad (3)$$

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 ($f_{conifer}$ =0.40, $f_{broadleaf}$ =0.42).

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

Tree species with IV>10% were determined as dominant.

Nitrogen and P stoichiometry (N and P concentration and N: P) of tree layer 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:

$$S_l = C_l B_l \qquad (6)$$

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:

$$S_T = \sum_{i=1}^{n} E_i B_i D_i \qquad (7)$$

where, S_T is the soil nutrient storage (t ha^{-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).

The relationships between foliar N and P and N: P in the tree layer, foliar N and P, N: P in the tree layer 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. tabuliformis*, *P. armandii*, and *Q. aliena* var.*acuteserrata*), most of the species were shade-tolerant (*Toxicodendron vernicifluum*, *Carpinus turczaninowii*) or neutral with respective to light (*Acer mono*, *Sorbus folgneri*) (Table 1). The dominant tree species with importance values >10% were *P. tabuliformis*, *P. armandii*, and *Q. aliena* var.*acuteserrata* (Table 1).

Commented [AOA8]: Insert space between RH(%) i.e. RH (%) etc on Table 1.

The IV (importance value) of big timber rate ~~of~~ for *P. tabuliformis*, *P. armandii*, and *Q. 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 tree layer

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. tabuliformis* and *P. armandii* were less than that in *Q. aliena* var. *acuteserrata* (Table 3). Moreover, foliar stoichiometric variables of the tree layer were also less than those in individual tree species in the mixed pine-oak stand (Table 3).

In the tree layer, concentrations of foliar N and P were significantly, positively correlated (p<0.05) with each other (Figure 1(a)). The Log foliar N concentration was significantly, positively correlated with the log N: P (Figure1 (b)). Foliar P concentration and N: P were significantly, negatively correlated (p<0.05) with each other (Figure1 (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, N: P, and concentrations of N and P were far higher in litter than in mineral soil (Table 4).

Formatted: Font color: Red

Commented [AOA9]: Applies to Line 32.

Formatted: Font color: Red

Commented [AOA10]: Verify the accuracy of your calculator. I got 9.87

Commented [AOA11]: Is IV in this work "importance value" or "important value"? Foot note of Table 3 must be consistent with manuscript text.

Commented [AOA12]: For clarity, use ".. mathematical log of foliar N...." to distinguish tree log from mathematical log. This is more so when it involves foliage but notice that log N:P is perfectly okay as in Line 204

Commented [AOA13]: I would have been surprised if this was not the case.

Having said that, no statistical analysis was shown on the Table to justify the claim. What are the statistical p-values?

Relationships ~~among~~ between N and P concentrations in litter and mineral soil were complex and none was ~~mostly non~~significant (Figure 2(a)-(f)). 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(d)). 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 (b)). Soil P concentration showed an exponential relationship with litter P concentration (Figure 2(e)). 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(c)). Phosphorus concentration of mineral soil increased linearly with litter P concentration (Figure 2(f)).

Relationship between foliar N and P concentration in the tree layer and mineral soil

At 0–10 cm depth, foliar N concentration in the tree layer was significantly, positively correlated (p>0.05) with N concentration of mineral soil (Figure 3(a)). In contrast, foliar P concentration in the tree layer showed a significant, cubic relationship with P concentration of mineral soil (p<0.05) (Figure 3(d)). Although foliar N and P concentrations in the tree layer were not significantly correlated with mineral soil at 11–20 cm depth, foliar N concentration in the tree layer increased with N concentration of mineral soil (Figure 3(b) and (e)). There was no relationship between foliar N concentration in the tree layer and N concentration of mineral soil at 21–30 cm depth (Figure 2(c)). Foliar P concentration in the tree layer increased with P concentration of mineral soil at 21–30 cm depth (Figure 2(f)).

DISCUSSION

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Commented [AOA14]: I was unable to open the figures of this manuscript but from the little I could glean off the pdf document I received from the Editor, it seems Fig. 2(d) has litter nitrogen on the X-axis and not P. If this is correct, the figure and manuscript need revision to address this finding.

Commented [AOA15]: The problem with Fig 2(d) applies to Fig 2(e)

Commented [AOA16]: I do not see the increased relationship with N. The relationship is spread through the sampling area and show no increase or decrease with depth or N concentration.

Commented [AOA17]: No litter P was analyzed in Fig. 2. All x-axis analyses are nitrogen. See 2a-2f.

Commented [AOA18]: Make sure that the correct figures are used in this research. It seems that Fig 3a has p=0.28 and not p>0.05.

Commented [AOA19]: Fig 3e shows foliar and soil P, not nitrogen. Revise sentence to reflect this. As the sentence is currently stated, it seems figures 3b and 3e are about foliar N increases with soil N. Only 3b is; 3e is not.

Commented [AOA20]: Although these are important findings, it is important to state clearly that none of the stoichiometry of N and P in relation to tree layer, and mineral soil was significant.

Foliar N and P stoichiometric traits in the tree layer in mixed pine-oak stand

The positive correlation between foliar N and P concentrations in the tree layer 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 tree layer were lower than both Chinese and global terrestrial plants. Similarly, foliar N concentration of *P. tabuliformis* and *P. armandii*, and foliar P concentration of *P. armandii* 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 the secrucial 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 *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 and P in *Q. aliena* var. *acuteserrata* was higher than that reported for Chinese and global mean levels. Foliar P concentration of *P. tabuliformis* was higher than the Chinese

Commented [AOA21]: Where are these positive correlations? No statistical p-values support it from your figures. The trend lines can point up or down but that does not mean positive or negative agreement when it is not statistically supported.

Commented [AOA22]: ……, and that these critical factors prevented …..

mean, but lower than the global mean. The growing period (from mid-May to late-September) in the study area was relatively short for ~~elevation of the~~ plots on high elevation (more than 1600 m) (Hou et al. 2017). 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 tree layer to soil nutrients

The N and P concentrations in mineral soil were generally low. 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}); 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 relationships between foliar N and P concentrations in the tree layer and mineral soil at various depths were ~~nonsignificant~~ not significant, and soil nutrients did not explain more than 14% of foliar nutrients in the tree layer. 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 tree layer. 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

Formatted: Font color: Red
Formatted: Font color: Red
Formatted: Font color: Red
Formatted: Font color: Red

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 in the tree layer.

Nutrient limitation of the tree layer

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 tree layer in the mixed pine-oak (Table 3) stand showed N limitation according to above mentioned criteria (Aerts and Chapin 1999; Koerselman 1996).

Formatted: Font color: Red

The relationships between soil nutrient storage and foliar N: P of the tree layer indicated that foliar N: P of the tree layer was correlated with soil N storage (Figure 4(a)). One possible reason may be that the growth of the tree layer was limited by N, and foliar N: P of the tree layer 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 tree layer, and foliar N: P of the tree layer 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 tree layer.

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

303 Chapin 1999; Koerselman 1996), 1.49 t ha^{-1} pure N should be used to fertilize forest land to
304 relieve N limitation on tree growth.

305 **Limitations of the analysis**

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

319 ## CONCLUSIONS

320 Our results indicated that N and P concentrations were low in leaves in the tree layer and
321 mineral soil, but high in litter. Concentrations of N and P in mineral soil were insufficient to
322 explain the variation in foliar N: P in the tree layer. However, N storage in mineral soil at 0–30
323 cm depth was correlated with foliar N: P. The growth of the tree layer was limited by N;
324 therefore, 1.49 t ha^{-1} pure N should be added to forest land to relieve this limitation. We further
325 recommend that appropriate fertilization rates should be included in the process of big timber
326 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.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by National Key Research and Development Program of China (No. 2017YFD0600504-2) from Ministry of Science and Technology of the People's Republic of China. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

National Key Research and Development Program of China: 2017YFD0600504-2.

Competing Interests

The authors declare that they have no competing interest.

Author Contributions

- Lin Hou conceived and designed the experiments, and wrote the paper.
- Zhenjie Dong calculated importance values of tree species and analyzed nutrients.
- Yuanyuan Yang and Z.D. analyzed the data.
- Shengli Zhang and Shuoxin Zhang reviewed the paper.

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, Pendall 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: curruent 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.

376 *Ecology Letters* **16**:764-772.
377 **Finn D, Page K, Catton K, Kienzle M, Robertson F, Armstrong R, and Dalal R. 2016**.
378 Ecological stoichiometry controls the transformation and retention of plant-derived
379 organic matter to humus in response to nitrogen fertilisation. *Soil Biology and*
380 *Biochemistry* **99**:117-127.
381 **Güsewell S. 2004**. N : P ratios in terrestrial plants: variation and functional significance. *New*
382 *Phytologist* **164**:243-266.
383 **Güsewell S, Koerselman W, and Verhoeven JTA. 2003**. Biomass N: P ratios as indicators of
384 nutrient limitation for plant populations in wetlands. *Ecological Applications* **13**:372-384.
385 **Gao Y, Yu G, and He N. 2013.** Equilibration of the terrestrial water, nitrogen, and carbon
386 cycles: advocating a health threshold for carbon storage. *Ecological Engineering*
387 **57**:366-374.
388 **Gundersen P, Sevel L, Christiansen JR, Vesterdal L, Hansen K, and Bastrup-Birk A.**
389 **2009**. Do indicators of nitrogen retention and leaching differ between coniferous and
390 broadleaved forests in Denmark? *Forest Ecology and Management* **258**:1137-1146.
391 **Han W, Fang J, Guo D, and Zhang Y. 2005**. Leaf nitrogen and phosphorus stoichiometry
392 across 753 terrestrial plant species in China. *New Phytologist* **168**:377-385.
393 **He JS, Fang J, Wang Z, Guo D, Flynn DFB, and Geng Z. 2006**. Stoichiometry and
394 large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China.
395 *Oecologia* **149**:115-122.
396 **He JS, Wang L, Dan FBF, Wang X, Ma W, and Fang J. 2008**. Leaf Nitrogen:Phosphorus
397 stoichiometry across Chinese grassland biomes. *Oecologia* **155**:301-310.
398 **Hong J, Wang X, and Wu J. 2015**. Effects of soil fertility on the N:P stoichiometry of
399 herbaceous plants on a nutrient-limited alpine steppe on the northern Tibetan Plateau.
400 *Plant and Soil* **391**:179-194.
401 **Hou L, Lei R, Zhang S, and Liu J. 2010**. Temporal and spatial variations of soil respiration in
402 *Pinus tabulaeformis* forest at Huoditang forest zone in the Qinling Mountains,China.
403 *Acata Ecologica Sinica* **30**:5225-5236.

404 **Hou L, Sun S, Liang L, Liang G, and Jiang L. 2017**. Effects of selective thinning and
405 residue removal on ground layer structure and diversity in a mixed pine–oak stand of the
406 Qinling Mountains, China. *Australian Journal of Botany* **65**:485-496.
407 **Knoll LB, McIntyre PB, Vanni MJ, and Flecker AS. 2009.** Feedbacks of consumer nutrient
408 recycling on producer biomass and stoichiometry: separating direct and indirect effects.
409 *Oikos* **118**:1732-1742.
410 **Koerselman W. 1996**. The vegetation N:P ratio: a new tool to detect the nature of nutrient
411 limitation. *Journal of Applied Ecology* **33**:1441-1450.
412 **National Soil Survey Office of China. 1998**. *Soils of China*. Beijing, China: Chinese
413 Agriculture Press.
414 **Reich PB, and Oleksyn J. 2004**. Global patterns of plant leaf N and P in relation to
415 temperature and latitude. *Proceedings of the National Academy of Sciences of the United*
416 *States of America* **101**:11001-11006.
417 **Rong Q, Liu J, Cai Y, Lu Z, Zhao Z, Yue W, and Xia J. 2015**. Leaf carbon, nitrogen and
418 phosphorus stoichiometry of *Tamarix chinensis* Lour. in the Laizhou Bay coastal wetland,
419 China. *Ecological Engineering* **76**:57-65.
420 **Sardans J, Rivas-Ubach A, and Peñuelas J. 2011**. Factors affecting nutrient concentration
421 and stoichiometry of forest trees in Catalonia (NE Spain). *Forest Ecology and anagement*
422 **262**:2024-2034.
423 **Sardans J, Rivasubach A, and Peñuelas J. 2012**. The elemental stoichiometry of aquatic and
424 terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem
425 structure and function: a review and perspectives. *Biogeochemistry* **111**:1-39.
426 **Scharler UM, Ulanowicz RE, Fogel ML, Wooller MJ, Jacobson-Meyers ME, Lovelock**
427 **CE, Feller IC, Frischer M, Lee R, McKee K, Romero I C , Schmit J P, Shearer C.**
428 **2015**.
429 Variable nutrient stoichiometry (carbon:nitrogen:phosphorus) across trophic levels
430 determines community and ecosystem properties in an oligotrophic mangrove system.
431 *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.

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.