



Effects of nutrient addition on foliar phosphorus fractions and their resorption in different-aged leaves of Chinese fir in subtropical China

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Abstract

Background and aims Anthropogenic activities have increased nitrogen (N) and phosphorus (P) inputs to terrestrial ecosystems, which may significantly alter P cycle through accumulation and resorption.

Methods We measured the concentrations of four different P fractions (inorganic, nucleic, sugar, and residual P) in both live leaves and senescent leaves in N and P additions in an evergreen plantation forest of subtropical China.

Results Adding moderate N plus P increased total, inorganic, and sugar P concentrations, which sustained the

leaf N/P balance and alleviated P limitation in Chinese fir (*Cunninghamia lanceolata*) plantation. Nitrogen and P (total, nucleic, and residue P) resorption proficiencies did but P (each of various fractions) resorption efficiency did not respond to nutrient addition. The concentrations and resorption efficiencies of most P fractions were lower in the old than young leaves, but their resorption proficiencies except for sugar P weren't different.

Conclusions Internal P cycles of Chinese fir could be strongly altered through increasing accumulation of inorganic P and sugar P fractions in response to soil P

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enrichment when combined with suitable N addition. The easily degradable P (inorganic and sugar P) were preferentially resorbed regardless of nutrient addition. Leaf age was a key-factor influencing the resorption extent of P fractions in Chinese fir.

Keywords Fertilization · Leaf traits · Forest trees · Nitrogen deposition · Nutrient resorption · Phosphorus

Introduction

Anthropogenic activities have increased nitrogen (N) and phosphorus (P) deposition, which may alter nutrient availability to plant growth and affect fundamental ecological processes. It has been estimated that N and P deposition were approximately 20–30 kg N hm⁻² year⁻¹ and 0.3–0.4 kg hm⁻² year⁻¹ in subtropical forest ecosystems, respectively (Zhu et al. 2015, 2016). Higher N inputs may also cause nutrient imbalances and affect the availability of other nutrient elements in subtropical forests. Previous studies have demonstrated that increased N input would decrease soils available P due to induce soil acidification (Liu et al. 2011; Zhang et al. 2018; Zheng et al. 2017), or increase soil P availability and foliar P concentrations because of the enhanced P-mineralizing enzymes activities (Marklein and Houlton 2012). Likewise, P addition could increase soil nitrification rate and/or N₂ fixation rate, resulting in the increased overall N availability for plant in P-poor soil (Reed et al. 2007; Cheng et al. 2018). There is a broad range of studies on how N and P additions affect P cycle (Vitousek et al. 2010; Huang et al. 2016), however, how different P fractions and their resorption in leaves respond to N and P inputs remains unclear (Yan et al. 2015), especially in the subtropical forest (Yang 2018).

Phosphorus is an essential nutrient that limits plant activity and primary productivity in forests (Cleveland et al. 2002; Li et al. 2016a, b). Foliar P may be functionally divided into four fractions: (1) Inorganic P is an easily soluble fraction which is used for cell metabolism. (2) Sugar P is referred as intermediates of carbon metabolism including Calvin cycle and glycolysis and plays an important role in material and energy metabolisms (Zhang et al. 2018). (3) Nucleic P (containing RNA and DNA) is involved in the reproduction of genetic information and protein synthesis (de Oliveira et al. 2018) and (4) Residual P is insoluble P compounds, which contains the phosphoproteins and

phospholipids that cannot be extracted or contributed to cell membrane (Hidaka and Kitayama 2011). Relatively few studies have investigated the response of plant P fractions to environmental alteration, such as elevated soil nutrient supply (Mayor et al. 2014; Tsujii et al. 2017). Inorganic P of many plant species including trees comprised 50–95% of the total P in fertilized gardens, but the inorganic P concentration of mature leaves of all taiga trees measured under condition of P deficiency only ranged between 12 and 25% (Chapin and Kedrowski 1983). Hidaka and Kitayama (2011) investigated 21 tropical tree species and found that foliar P concentration was reduced in soils of P limitation due to the reduction of the concentration of inorganic P and nucleic P. Among the four P fractions, inorganic P may be easily withdrawn from senescence leaves and other P fractions need to be degraded and then resorbed (Hidaka and Kitayama 2011). However, how these foliar P fractions respond to altered soil nutrient availability is not well studied, especially in subtropical forests. Therefore, investigating the effect of nutrient addition on the accumulation and resorption of each leaf P fraction can improve our understanding of nutrient limitation in terrestrial ecosystems.

Nutrient resorption, a mechanism by which plants withdraw the nutrient elements from senesced leaves into living tissues, is an important nutrient conservation strategy that could be described by nutrient resorption efficiency and proficiency (Aerts 1996; Killingbeck 1996). Nutrient resorption from senescing leaves is related to soil nutrient supply, and the global average P resorption efficiency in conifers tree was reported as 64.9% (Vergutz et al. 2012). Plants regulated the variation of P resorption efficiency through selectively degrading organic compounds depending on environmental conditions. Previous study showed that lower resorption efficiency of inorganic P in P-rich site with the excessive inorganic P remained in senescent leaves (Ostertag 2010). However, P resorption efficiency approached 82% on sites with severe P deficiency because of the withdrawn of inorganic P along with the P mobilized from hydrolyzed lipids and nucleic acids prior to abscission (Denton et al. 2007; Lambers et al. 2015a). These studies suggested that the resorption of different P fractions depended on plant-soil P supply.

For evergreen tree species, leaf age is a key factor controlling plant nutrient accumulation and resorption. On the one hand, foliar nutrient concentration generally declines with leaf age because the shading provided by

younger leave reduces light availability and downregulate the photosynthetic rates and nutrient accumulation in older leaves (Warren 2006; Chen et al. 2015). On the other hand, nutrient resorption efficiency for different-aged leaves implies a xylem or phloem transport capacity of nutrients by live tissues of plants, which is expected to decline with leaf age in coniferous trees (Yuan et al. 2017). Despite many studies explored the relationship between nutrient resorption and foliar or soil nutrient concentrations, how the resorption of different P functional fractions changes in different-aged leaves remains poorly known (Lü et al. 2016).

Chinese fir (*Cunninghamia lanceolata*) is one of the most important plantation tree species in southern China since it generally grows rapidly in subtropics. Meanwhile, the tree species provides an excellent experimental model system to study the responses of different-age leaves to nutrient addition because it is an evergreen conifer whose leaves and twigs grow and senesce at the same time (Li et al. 2016a, b). Our previous study reported that nutrient addition during the first year affected the total nutrient concentrations and their resorption of different-aged leaves in Chinese fir plantation to explore the nutrient limitation in subtropical plantation (Chen et al. 2015). Recently, Turner et al. (2018) found species-specific P limitation was pervasive in tropical forests since some tree species can grow rapidly in the environment with extremely low P availability. However, the mechanism that trees promote the P use efficiency remains unclear. In this study, we conducted a continuous nutrient addition experiment for five years to identify the responses of various P functional fractions and their resorption in different-aged leaves of Chinese fir in order to understand the potential adaptation of Chinese fir to P-poor environment in subtropical forests. The specific objectives of our study were to answer the following three key questions: 1) how do different foliar P fractions respond to N and P addition treatments? 2) how do N and P addition treatments affect the resorption efficiency and proficiency of different foliar P fractions, and which P fraction was most preferentially resorbed in response to altered soil nutrient availability? 3) do different foliar P fractions and their resorption respond differently to N and P additions between young and old leaf? We expect that our study results would provide knowledge necessary for developing an improved nutrient conservation and management practice in Chinese fir plantations.

Materials and methods

Study region

The experimental site was conducted in the Qianyanzhou Ecological Research Station, Chinese Academy of Sciences, Taihe County, Jiangxi Province of Southern China (26°42'N, 115°04'E). The site is characterized by a red, nutrient-poor soil, and a subtropical moist monsoon climate. The average annual precipitation is 1600 mm and the average annual temperature is 17.9 °C. The mean air temperature and precipitation at the site in July were 29.7 °C and 171.0 mm, respectively. The soil is classified as Ultisols (locally called 'red soil'). The average height of Chinese fir plantation is 15 m and diameter at breast height is about 13 cm (Tang et al. 2016).

Experimental treatments

We selected plot with N and P addition treatment in 12-year-old Chinese fir plantation. Six treatments and five replications were established on five separate hilly slopes and set up in 2011 using the randomized complete block design (See Fig. S1, Chen et al. 2015). The six treatments included: +N1 (50 kg N ha⁻¹ year⁻¹), +N2 (100 kg N ha⁻¹ year⁻¹), +P (50 kg P ha⁻¹ year⁻¹), +N1 + P (50 kg N plus 50 kg P ha⁻¹ year⁻¹), +N2 + P (100 kg N plus 50 kg P ha⁻¹ year⁻¹) and CK (control, without N and P addition). Each of the 30 plots was 20 × 20 m in size, separated by buffer zones at least 20 m on each side, was randomly arranged within each replication. Nitrogen and P were added as NH₄NO₃/CO(NH₂)₂ and NaH₂PO₄, respectively. The fertilizer was weighed and mixed with 8 kg of clean and dry fine sand and applied to each plot zone on non-rainy days for four times each year (in March, June, September and December). The control plots received fine sand without N and P addition (See Chen et al. 2015).

Sample collection

During the growing season in 2016, three representative trees base on average diameter at breast height (DBH) and height were selected to collect soil and leaf tissues for each plot. In August 2016, living roots were carefully taken out from the southern to northern transect under each tree canopy and hand-shaken gently to remove loose soil. The soil strongly adhering within 4 mm of the roots after gentle hand-shaking was considered as

rhizosphere soil, and the remaining soil was considered bulk soil (Hu et al. 2017). The collected rhizosphere and bulk soil samples were combined into one soil sample per plot, respectively. The rhizosphere and bulk soils were immediately brought back to the laboratory and stored in refrigerator at 4 °C. The needle leaves of Chinese fir were stored in a freezer before being microwaved at 600 W for 90 s to stop all enzymatic activity, oven dried for 72 h at 65 °C and ground to a fine powder (Li et al. 2018). In July 2016, we selected three representative trees based on the mean DBH and height in each plot. The south-faced branch in the middle section of the canopy of representative trees was collected. The first order of branching is young leaves (<1-year old), and the second or third orders of branching are old leaves (>1-year old). The leaves with the same age were mixed together to obtain a composite sample for each plot. In addition, leaf litters of Chinese fir were collected from November 2016 to March 2017 using 1 m × 1 m nylon mesh traps under the canopies of three representative trees within each plot. Since Chinese fir litter generally fall down as a whole branch, we used the same method described above to assemble a composite sample for young leaf and old leaf litters.

Chemical analysis

Total N was determined by the Kjeldahl method, while leaf and soil P concentration was determined by ultraviolet spectrophotometer after the samples were digested with H₂SO₄ (Allen 1989). We divided foliar P into four fractions: inorganic P, sugar P, nucleic P and residual P. The fractionation procedure was based on the following methods as in Close and Beadle (2004). Oven-dried samples (0.4 g) were extracted initially in 50 mL of 0.3 M Trichloroacetic acid (TCA) at 4 °C for 1 h, with shaking every 10 min. The extraction process was conducted twice, use 25 ml of 0.3 M Trichloroacetic acid for each time. This was followed by centrifuging (Low Speed Centrifuge, Yineng, TDL-5A, China), and then mixed the supernatant, one part of supernatant removal for inorganic P measured. A sub-sample (20 mL) of the supernatant from each of the cold was evaporated to dryness at 100 °C and the digested with 5 mL H₂SO₄ for sugar P measure which is calculate the difference between total cold P and inorganic P. And the residue remaining was extracted with 0.15 M TCA at 90 °C for 1 h. A sub-sample (20 mL) of the supernatant from each of the hot extraction procedures was evaporated to

dryness at 100 °C and digested with 5 mL H₂SO₄ for nucleic P measure. These P fractions were determined with the molybdenum blue spectrophotometric. The residual P was total P minus inorganic P, sugar P and nucleic P.

Both rhizosphere and bulk fresh soils were analyzed for available nutrients. The NH₄⁺-N and NO₃⁻-N were extracted with 2 M KCl solution, separately measured by spectrophotometry following filtration using the indophenol blue method and the cadmium reduction method after soil was shaken in shaking table (JIUPIN-COS-100C, China) for half an hour. Mineral N was NH₄⁺-N plus NO₃⁻-N. Available P was extracted by 0.5 M NaHCO₃ solution at room temperature of about 25 °C for 0.5 h and determined by the ultraviolet spectrophotometer after the soil was shaken (Olsen 1954).

Nutrient resorption efficiency and proficiency

Nutrient resorption efficiency was estimated as the percent reduction between nutrient concentrations in green and senesced leaves (Aerts 1996). Nutrient resorption proficiency is defined as the terminal nutrient concentration in senesced leaves (Killingbeck 1996). Thus, nutrient resorption proficiency was calculated as the mass-based nutrient concentration in senesced needles, while the nutrient resorption efficiency was calculated with formula: Nutrient resorption efficiency = [(nutrient in living leaf – nutrient in the litter) / (nutrient in living leaf)] × 100%. To detect age-related difference, we determined the nutrient resorption proficiency and efficiency separately for young and old leaves.

Data analyses

All statistical analyses were performed with IBM SPSS 19.0 (SPSS, Chicago, IL, USA). Before statistical analysis, the normality of data was tested by Levene's test, and all non-normal data were transformed to follow a normal distribution. One-way ANOVA was used to test the effect of nutrient addition on soil available nutrients, while two-way ANOVA was used to test the effect of nutrient addition and leaf age on foliar nutrient concentration, and nutrient resorption efficiency and proficiency. Since nutrient addition treatment did not interact with the effect of leaf age for each dependent variable examined in our study (see Table S1), we examined each factor independently. Thus, we pooled young and old leaves to obtain the averages of leaf nutrient variables when assessing the

effect of nutrient addition treatment. Tukey's HSD test was used to determine the significant difference in soil available nutrient, foliar nutrient concentration, foliar nutrient resorption efficiency and proficiency among different treatments. The paired t-test was used to compare the differences in nutrient variables between young and old leaves. Correlation analysis was used to examine the relationships among foliar P fractions and total N, P concentrations, and N/P ratio in different-aged leaves, and between rhizosphere nutrient supply and leaf nutrient variables.

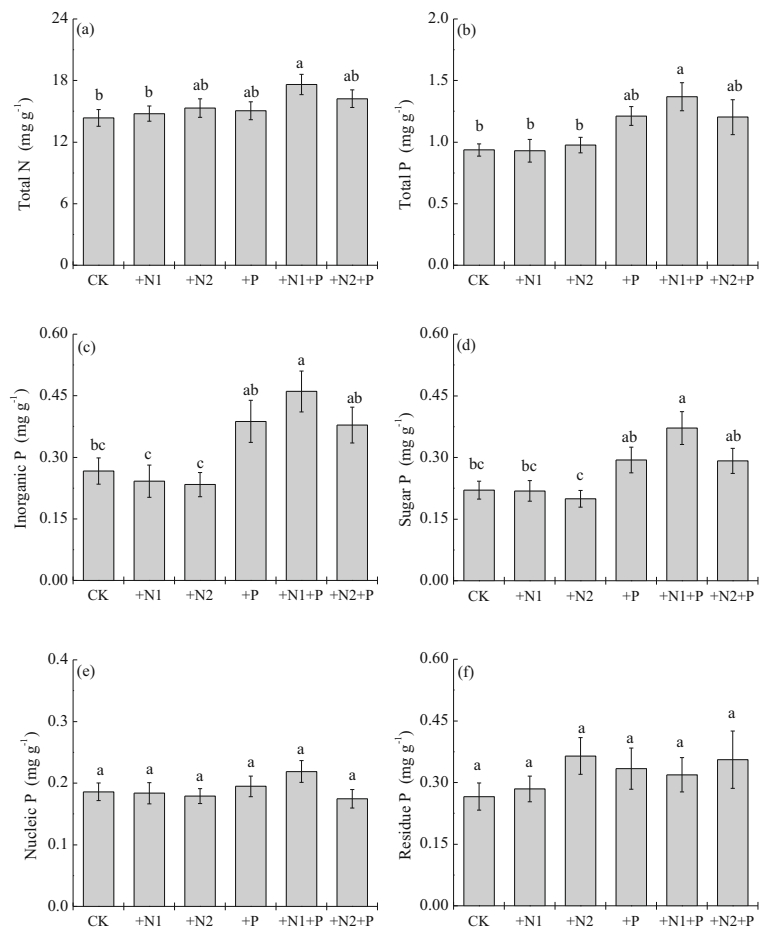
Results

Foliar nutrients and their resorption in response to exogenous nutrient addition

Total N and P concentrations and N/P significantly differed among the six nutrient addition treatments

Fig. 1 Effects of exogenous nutrient additions on the concentrations of total N, total P and various P fractions in living leaves (pooled with young and old leaves) of Chinese fir.

Note: +N1: 50 kg N ha⁻¹ year⁻¹, +N2: 100 kg N ha⁻¹ year⁻¹, +P: 50 kg P ha⁻¹ year⁻¹, +N1 + P: 50 kg N plus 50 kg P ha⁻¹ year⁻¹, +N2 + P: 100 kg N plus 50 kg P ha⁻¹ year⁻¹ and control: without N and P addition. Different letters above the bars (standard error) indicate significant differences ($P < 0.05$) among the treatments



(Table S1). The combined N and P addition treatment (+N1 + P) resulted in 22.7% higher foliar N than CK (Fig. 1a) and 46.2% higher foliar P concentration than CK (Fig. 1b). The two N addition only (+N1 and +N2) treatments resulted in higher N/P than the P addition only (+P) and +N1 + P treatment while +P resulted in lower N/P than CK and +N2 + P (Fig. S2a).

Among the four P fractions, the concentrations of inorganic P and sugar P differed among the six nutrient addition treatments while the concentrations of nucleic P and residue P did not (Table S1). Compared to CK, only +N1 + P resulted in higher inorganic P and structural P concentrations (Fig. 1c and d). However, P addition alone (+P) or combined with N addition (+N1 + P and +N2 + P) all resulted in higher inorganic P and sugar P concentrations than the two N addition only (+N1 and +N2) treatments (Fig. 1c and d). When expressed as percentage of the total P, only inorganic P was affected by nutrient addition treatment. Adding P alone or in combination with N (+N1 + P and +N2 + P)

significantly increased the proportion of inorganic P compared to the two N addition only (+N1 and +N2) treatments (Fig. S3).

The resorption proficiency differed among the six nutrient addition treatments for total N, total P, nucleic P and residue P, but not for inorganic P and sugar P (Table S1). Adding both P and N also resulted in higher total N resorption proficiency than CK and N addition alone (+N1 and +N2) treatments (Fig. 2a). Adding P alone (+P) or in combination with N (+N1 + P and +N2 + P) increased total P resorption proficiency compared to CK and N addition only (+N1 and +N2) treatments (Fig. 2b). Similarly, +N1 + P treatment resulted in higher nucleic P resorption proficiency (Fig. 2e) while adding both P and N (+N1 + P and +N2 + P) resulted in higher residue P proficiency. In contrast, there was no significant difference in the resorption efficiency among the six nutrient addition treatments for total N, total P, and each of the four P fractions (Table S1 and Fig. S4). Further, the ratio of N resorption proficiency to P

resorption proficiency (NRP/PRP) was higher for the higher level N addition (+N2) treatment compared with other treatments (Fig. S2b), but the ratio of N resorption efficiency to P resorption efficiency (NRE/PRE) did not change among the nutrient addition treatments (Fig. S2c).

Foliar nutrients and their resorption in response to leaf age

Total N and P concentration in young leaves were 22.74% and 37.63% lower than old leaves, respectively. While N/P increased with leaf age (Table 1). Inorganic P, sugar P and nucleic P were also 60.0%, 40.9%, 37.5% declined with leaf age, respectively (Table 1). When expressed as percentage of the total P, leaf age affected both inorganic P and residue P but not sugar P and nucleic P. The proportion of inorganic P declined, while the proportion of residue P increased with leaf age (Table 1).

There was no difference in nutrient resorption proficiency found for total N, total P, and each of the four P

Fig. 2 Effects of exogenous nutrient additions on the resorption proficiencies of total N, total P and various P fractions in leaves (pooled with young and old leaves) of Chinese fir. Note: +N1: 50 kg N ha⁻¹ year⁻¹, +N2: 100 kg N ha⁻¹ year⁻¹, +P: 50 kg P ha⁻¹ year⁻¹, +N1 + P: 50 kg N plus 50 kg P ha⁻¹ year⁻¹, +N2 + P: 100 kg N plus 50 kg P ha⁻¹ year⁻¹ and control: without N and P addition. PRP: P resorption proficiency, NRP: N resorption proficiency, IPRP: inorganic P resorption proficiency, SPRP: sugar P resorption proficiency, NPRP: Nucleic P resorption proficiency, RPRP: Residue P resorption proficiency. Different letters above the bars (standard error) indicate significant differences ($P < 0.05$) among the treatments

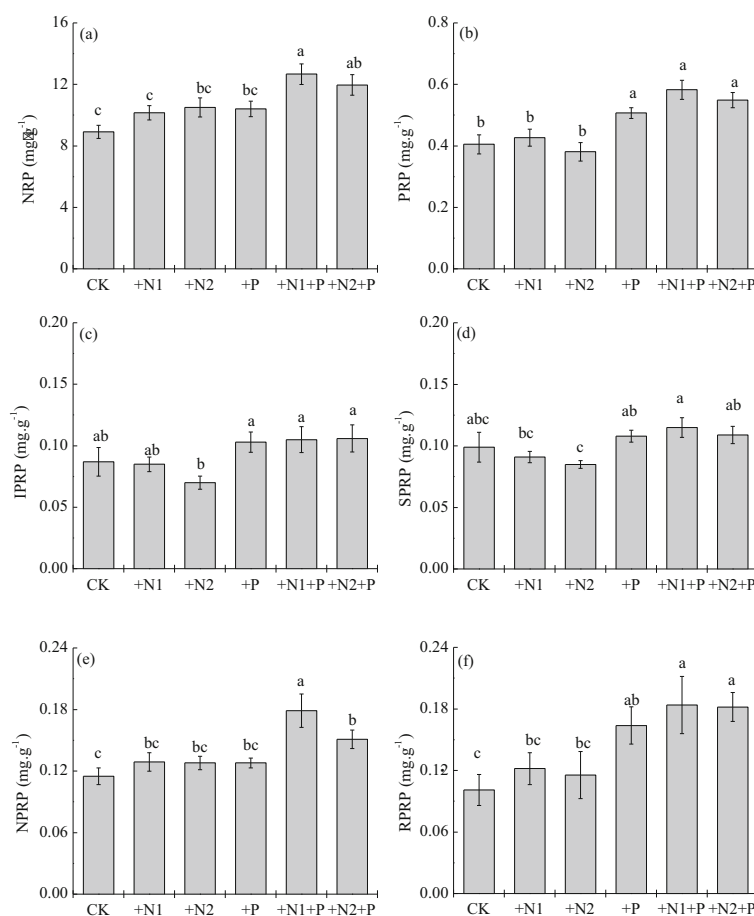


Table 1 Nutrient concentration, resorption proficiency and resorption efficiency in young versus old leaves of Chinese fir, with their differences analyzed by T-test

Variable	Young leaves	Old leaves	T-test
Total N concentration (mg g ⁻¹)	17.16 ± 0.45	13.98 ± 0.41	<i>P</i> < 0.05
Total P concentration (mg g ⁻¹)	1.28 ± 0.06	0.93 ± 0.04	<i>P</i> < 0.05
Inorganic P concentration (mg g ⁻¹)	0.41 ± 0.03	0.25 ± 0.02	<i>P</i> < 0.05
Sugar P concentration (mg g ⁻¹)	0.31 ± 0.02	0.22 ± 0.01	<i>P</i> < 0.05
Nucleic P concentration (mg g ⁻¹)	0.22 ± 0.01	0.16 ± 0.01	<i>P</i> < 0.05
Residual P concentration (mg g ⁻¹)	0.33 ± 0.03	0.31 ± 0.03	ns
Inorganic P proportion (%)	31.10 ± 1.01	26.57 ± 1.23	<i>P</i> < 0.05
Sugar P proportion (%)	24.21 ± 0.67	23.61 ± 1.20	ns
Nucleic P proportion (%)	18.25 ± 0.73	17.21 ± 0.93	ns
Residual P proportion (%)	26.44 ± 1.53	32.60 ± 2.49	<i>P</i> < 0.05
Total N resorption proficiency (NRP, mg g ⁻¹)	10.97 ± 0.43	10.57 ± 0.35	ns
Total P resorption proficiency (PRP, mg g ⁻¹)	0.49 ± 0.02	0.46 ± 0.02	ns
Inorganic P resorption proficiency (mg g ⁻¹)	0.10 ± 0.01	0.09 ± 0.01	ns
Sugar P resorption proficiency (mg g ⁻¹)	0.11 ± 0.01	0.09 ± 0.00	<i>P</i> < 0.05
Nucleic P resorption proficiency (mg g ⁻¹)	0.14 ± 0.01	0.14 ± 0.01	ns
Residual P resorption proficiency (mg g ⁻¹)	0.14 ± 0.01	0.15 ± 0.01	ns
Total N resorption efficiency (NRE, %)	36.21 ± 2.27	24.32 ± 2.56	<i>P</i> < 0.05
Total P resorption efficiency (PRE, %)	61.04 ± 1.38	49.41 ± 2.19	<i>P</i> < 0.05
Inorganic P resorption efficiency (%)	73.48 ± 1.88	62.66 ± 2.59	<i>P</i> < 0.05
Sugar P resorption efficiency (%)	63.66 ± 1.99	53.09 ± 2.18	<i>P</i> < 0.05
Nucleic P resorption efficiency (%)	35.82 ± 3.23	13.64 ± 4.15	<i>P</i> < 0.05
Residual P resorption efficiency (%)	53.51 ± 4.14	50.03 ± 4.23	ns
N/P	13.92 ± 0.39	15.49 ± 0.59	<i>P</i> < 0.05
NRP/PRP	22.92 ± 0.69	23.70 ± 0.94	ns
NRE/PRE	0.61 ± 0.03	0.51 ± 0.04	<i>P</i> < 0.05

Values are the mean ± SE (n = 5), ns, not significant with *P* > 0.05

fractions except for sugar P between the young and old leaves. The resorption proficiency of sugar P declined with leaf age (Table 1). In contrast, total N and P resorption efficiency, and their ratio (NRE/PRE) declined with leaf age (Table 1). Except for the residue P, the resorption efficiency of the other three P fractions declined with leaf age (Table 1).

For the young leaves, both inorganic P and sugar P were positively correlated with total N and total P, and negatively correlated with N/P. Nucleic P was positively correlated with total N and total P. Residue P was positively correlated with total P, and negatively correlated with N/P (Table 2). For the old leaves, none of the four P fractions correlated with total N, but all fractions except nucleic P were positively correlated with total P and negatively correlated with N/P (Table 2).

Table 2 The correlation coefficients among foliar P fractions and total N, total P concentration, and N/P ratio in young and old leaves of Chinese fir

Tissues	Inorganic P	Sugar P	Nucleic P	Residual P
Young leaves				
Total N	0.73**	0.69**	0.65**	0.33 ^{ns}
Total P	0.92**	0.91**	0.47**	0.54**
N/P	-0.80**	-0.79**	-0.16 ^{ns}	-0.43*
Old leaves				
Total N	0.34 ^{ns}	0.29 ^{ns}	0.13 ^{ns}	0.30 ^{ns}
Total P	0.77**	0.61**	0.31 ^{ns}	0.57**
N/P	-0.54**	-0.43*	-0.25 ^{ns}	-0.40*

ns not significant with *P* > 0.05; *, *P* < 0.05; **, *P* < 0.01

The response of soil nutrient availability and their correlations with foliar nutrients and resorption

For rhizosphere soil, nutrient addition did not change soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations when compared with CK, but higher soil mineral N ($\text{NH}_4^+\text{-N}$ plus $\text{NO}_3^-\text{-N}$) concentration was observed in +N2 than in +P and +N2 + P (Table 3). For bulk soil, N addition at the higher level (+N2) resulted in higher concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and mineral N, when compared with CK and other four nutrient addition treatments (Table 3). For rhizosphere soils, P addition alone or in combination with N greatly increased soil available P concentration compared with CK and the two N addition only treatments (Table 3).

Regardless of leaf age, the concentrations of total P, inorganic P and sugar P, and the resorption proficiencies of total P, sugar P, nucleic P and residual P in both young and old leaves were positively correlated with rhizosphere soil available P, and negatively correlated with N/P. Meanwhile, rhizosphere soil mineral N was negatively correlated with PRP and residual P resorption proficiency, but positively correlated with NRP/PRP in young and old leaves (Table 4). In addition, rhizosphere soil mineral N was negatively correlated with inorganic P and sugar P concentrations, and positively correlated with N/P in young leaves, but not in old leaves. Rhizosphere soil P was positively correlated with total N concentration and residual P resorption proficiency, and negatively correlated with NRP/PRP in young but not in old leaves. In contrast, rhizosphere soil P was

positively correlated with inorganic P resorption proficiency and sugar P resorption efficiency in old but not in young leaves (Table 4).

Discussion

Foliar P fractions in response to exogenous nutrient additions

We found that N addition alone did not affect the concentration of leaf total P and each P fraction. This result was not surprising given that N addition did not affect soil P supply. The availability of soil P is often modified by microorganism through P mineralization, which would be affected by phosphatase enzyme activity (Dong et al. 2015). Our previous study showed that N addition did not affect the acid phosphatase enzyme in either understory plant rhizosphere or bulk soil in Chinese fir plantation (Wang et al. 2019). Some studies suggested that N addition generally resulted in higher leaf N concentration, which could promote plants to absorb more P to maintain plant nutrient balance (Lü et al. 2016). However, we did not detect an increase in leaf N concentration or leaf P concentration following N addition and leaf N and P concentration was weakly related with soil mineral N. Therefore, N addition did not aggravate P limitation of Chinese fir nutrient cycle in this area.

Interestingly, combined N and P addition treatment increased the foliar concentration of total P and two P

Table 3 Available nutrient in rhizosphere and bulk soils of Chinese fir plantations after nutrient addition treatment for 5 years

Variables	Control	+N1	+N2	+P	+N1 + P	+N2 + P
Rhizosphere soil						
$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	9.19 ± 1.72a	9.07 ± 2.05a	11.41 ± 2.14a	5.48 ± 0.63a	9.33 ± 2.93a	6.04 ± 0.58a
$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	4.60 ± 1.01a	5.59 ± 1.28a	6.48 ± 2.36a	3.35 ± 0.72a	4.63 ± 0.96a	4.38 ± 0.79a
Mineral N (mg kg^{-1})	13.79 ± 1.46ab	14.67 ± 1.85ab	17.89 ± 3.52a	8.83 ± 0.96b	13.96 ± 3.83ab	10.42 ± 0.71b
Available P (mg kg^{-1})	2.92 ± 0.28b	2.36 ± 0.23b	2.00 ± 0.11b	91.89 ± 23.11a	96.05 ± 15.02a	68.48 ± 16.05a
Bulk soil						
$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	4.32 ± 0.37b	3.84 ± 0.2b	6.60 ± 0.53a	4.12 ± 0.29b	4.66 ± 0.67b	4.04 ± 0.49b
$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	1.91 ± 0.73b	2.64 ± 0.35b	9.02 ± 3.66a	2.75 ± 0.97b	3.67 ± 1.35b	4.05 ± 1.25b
Mineral N (mg kg^{-1})	6.24 ± 0.95b	6.48 ± 0.36b	15.62 ± 3.85a	5.92 ± 1.71b	8.33 ± 1.96b	8.09 ± 1.3b
Available P (mg kg^{-1})	3.61 ± 2.38b	0.98 ± 0.08b	5.20 ± 4.42b	69.24 ± 18.59a	33.29 ± 20.03b	9.43 ± 3.92b

Values are the mean ± SE ($n = 5$). The different letters indicate significant differences ($P < 0.05$) among the treatments

+N1 50 kg N ha^{-1} year $^{-1}$; +N2 100 kg N ha^{-1} year $^{-1}$; +P 50 kg P ha^{-1} year $^{-1}$; +N1 + P 50 kg N plus 50 kg P ha^{-1} year $^{-1}$; +N2 + P 100 kg N plus 50 kg P ha^{-1} year $^{-1}$ and control: without N and P addition

Table 4 The correlations among rhizosphere soil nutrient availability and foliar concentration, resorption proficiency and resorption efficiency in the studied Chinese fir plantations

Rhizosphere soil	Mineral N	Available P	Mineral N	Available P
	Young leaves		Old leaves	
Chinese fir Leaves				
Total N concentration	-0.09 ^{ns}	0.45*	0.12 ^{ns}	0.27 ^{ns}
Total P concentration	-0.26 ^{ns}	0.50**	-0.09 ^{ns}	0.56**
Inorganic P concentration	-0.42*	0.50**	-0.17 ^{ns}	0.61**
Sugar P concentration	-0.45*	0.51**	-0.21 ^{ns}	0.59**
Nucleic P concentration	-0.12 ^{ns}	0.25 ^{ns}	0.22 ^{ns}	0.23 ^{ns}
Residual P concentration	0.23 ^{ns}	0.18 ^{ns}	0.04 ^{ns}	0.05 ^{ns}
Total N resorption proficiency (NRP)	-0.18 ^{ns}	0.24 ^{ns}	-0.14 ^{ns}	0.33 ^{ns}
Total P resorption proficiency (PRP)	-0.45*	0.58**	-0.37*	0.54**
Inorganic P resorption proficiency	-0.33 ^{ns}	0.23 ^{ns}	-0.09 ^{ns}	0.36*
Sugar P resorption proficiency	-0.32 ^{ns}	0.42*	-0.20 ^{ns}	0.40*
Nucleic P resorption proficiency	-0.26 ^{ns}	0.42*	-0.03 ^{ns}	0.38*
Residual P resorption proficiency	-0.34 ^{ns}	0.49**	-0.40*	0.36 ^{ns}
Total N resorption efficiency (NRE)	0.15 ^{ns}	0.09 ^{ns}	0.26 ^{ns}	-0.10 ^{ns}
Total P resorption efficiency (PRE)	0.26 ^{ns}	-0.08 ^{ns}	0.20 ^{ns}	-0.01 ^{ns}
Inorganic P resorption efficiency	-0.17 ^{ns}	0.30 ^{ns}	-0.31 ^{ns}	0.30 ^{ns}
Sugar P resorption efficiency	-0.24 ^{ns}	0.16 ^{ns}	-0.31 ^{ns}	0.43*
Nucleic P resorption efficiency	0.15 ^{ns}	-0.22 ^{ns}	0.11 ^{ns}	0.03 ^{ns}
Residual P resorption efficiency	0.55**	-0.34 ^{ns}	0.01 ^{ns}	-0.01 ^{ns}
N/P	0.38*	-0.42*	0.29 ^{ns}	-0.42*
NRP/PRP	0.46*	-0.58**	0.42*	-0.33 ^{ns}
NRE/PRE	0.04 ^{ns}	0.15 ^{ns}	0.15 ^{ns}	-0.16 ^{ns}

^{ns} $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

fractions (i.e., inorganic P and sugar P). Previous studies reported that soil available P increased with P input, and N addition, when combined with P input, also simulated plant P absorption from the soil (Huang et al. 2016; Liu et al. 2017), which are consistent with our results. Inorganic P plays an important role in cell metabolic activity. Previous study suggests that the balance between the levels of inorganic P and phosphorylated intermediates may be regulated to maintain rates of photosynthesis when inorganic P is deficient (Stitt et al. 2010). The depletion of inorganic P in the cytosol and chloroplast leads to the reduction of inorganic P in the vacuole (Mimura 1995). Ostertag (2010) showed that fertilization experiments exponentially increased inorganic phosphorus in tree leaves, suggesting that excessive inorganic P was stored in vacuoles. The concentration of sugar P is mediated by enzymes, and sugar P increases with increasing enzyme abundance (Lambers et al. 2015a). Thus, combined N and P addition treatment can increase the availability of N required for

enzyme synthesis, which would increase sugar concentration. Nutrient addition treatment did not affect nucleic P that is allocated to rRNA for protein synthesis. This result suggested that nucleic P was not regulated by soil nutrient supply in our study, which is inconsistent with other studies that showed a decrease of protein synthesis rate with a lower P allocation to nucleic P in P-poor soil (Hidaka and Kitayama 2011). Consequently, the increased leaf P concentration following nutrient addition in our study may be attributed to the increase in the inorganic P and sugar P fractions in Chinese fir.

As expected, our results show that foliar N/P decreased with increasing foliar P concentrations due to P addition or combined P and N addition. Leaf N/P ratio can be used as a diagnostic tool for evaluate nutrient limitation in terrestrial ecosystems (Güsewell 2004; Lambers et al. 2008). Otherwise, combined P and N addition increased leaf N concentration and P concentration in our study indicated that the growth of Chinese fir might be co-limited by N and P, which was consistent with our

previous study (Chen et al. 2015). Phosphorus addition or combined P and N addition may alleviate P limitation by increasing foliar P concentrations. However, we found that foliar N/P was significantly related to inorganic P and sugar P in this study, which suggests that nucleic acid P, which was only slightly affected by nutrient condition, can meet requirement for rRNA synthesis. Tree species on P-poor soils reduce the demand for foliar P by reducing concentrations of both inorganic P and nucleic acid P, which may potentially limit growth and productivity (Hidaka and Kitayama 2011). Our results suggest that inorganic P and sugar P play important role in determining foliar N/P when available soil P is abundant, because the proportion of inorganic P and sugar P exceeds that of nucleic P in Chinese fir plantation. Therefore, N and P enrichment alleviate P limitation by increasing inorganic P and sugar P that may potentially promote growth and productivity of Chinese fir.

Phosphorus fractions resorption in response to exogenous nutrient additions

Phosphorus addition alone or in combination with N decreased foliar P resorption proficiency in our study, which are consistent with some previous studies (Feller et al. 2007; Mayor et al. 2014). Although the combined P and N addition did increase foliar P concentration, mostly by increasing inorganic P and sugar P, it also decreased the resorption proficiency of total P, mostly by decreasing the resorption proficiency of nucleic P and residue P. Our results showed that P resorption proficiency could be determined by the variation of nucleic P and residue P fraction concentration in senesced leaves. Nucleic P and residue P fraction in senesced leaves are much more immobile and less degradable than other P fractions during the process of resorption (Tsujii et al. 2017). In this study, the increase of P resorption proficiency in P addition and N addition combined with P addition treatments may be attributed to the increase of recalcitrant P compounds concentration in senesced leaf.

It has been widely reported that nutrient resorption efficiency would decline following the addition of the respective nutrient (e.g., Feller et al. 1999; Soudzilovskaia et al. 2007; Yan et al. 2015). However, P resorption efficiency did not respond to P addition (alone or combined with N addition) in our study. As a result, the nutrient resorption efficiency remained unaffected by nutrient addition treatments. However, a

previous study showed that tree species adapted to P deficiency environment expended more energy to degrade recalcitrant compounds to increase P resorption efficiency, but was not the case in P-rich soils (Tsujii et al. 2017). This result is inconsistent with our results that nutrient addition did not affect the resorption efficiency of P fractions. Therefore, P absorb rate of Chinese fir rather than P resorption was likely responsible for the increase in foliar P concentration due to the combined P and N addition. It was suggested that Chinese fir may expend less energy to obtain P from the soil than withdraw P from senesced leaves (Chen et al. 2015). Previous studies also reported that P addition increased both green and senesced leaf P concentrations in conifers while did not change P resorption efficiency (Yuan and Chen 2015; Yang 2018).

Our results show that the resorption of inorganic and sugar P fractions (easily degradable P) were higher than the resorption of nucleic and residue P fractions (recalcitrant P compounds). Among the four P fractions, inorganic P is expected to have higher resorption efficiency due to its high mobility. Similarly, sugar P is much more conveniently degraded compared to nucleic acids P and residue P, because sugar P is referred as intermediates of carbon metabolism that can be degraded relatively easily (Tsujii et al. 2017). In contrast, much more energy must be expended to degrade recalcitrant P compounds in senesced leaves to increase P resorption efficiency in P limited environment. Therefore, under soil nutrient supply enrichment, Chinese fir in the study regulated its P resorption efficiency without using recalcitrant P compounds.

In recent studies, the N: P resorption ratios (NRE/PRE) have been used to indicate nutrient limitations because it could be affected by changeable nutrient availability (Güsewell 2005; Reed et al. 2012). Our study found that NRE/PRE was <1.0 in leaves and did not respond to exogenous nutrient additions. It suggested that more P was consistently resorbed relative to N in Chinese fir plantation. The higher PRE than NRE in our study suggested that Chinese fir was generally limited by P availability in the study area. We found both NRE and PRE were not significantly correlated with soil available nutrient in our study, which supports that nutrient resorption efficiency might be independent of the status of that nutrient (Yang 2018). However, nutrient resorption proficiency might be more sensitive than resorption efficiency to nutrient availability (Killingbeck 1996). We found that nutrient addition did significantly affect NRP/PRP, which may accelerate

N and P cycling because nutrient proficiency is directly related with litter decomposition process and nutrient cycling (Wang et al. 2014).

Leaf age effects on P internal cycle

In this study, we found that both total P and each P fractions concentrations, except for residue P, were lower in the old than young leaves in Chinese fir (Table 1; Table S2). The difference may be caused by the following two plausible reasons. First, P could be transferred from older leaves to younger leaves (Wyka et al. 2016). Previous study shows that the regrowth of new leaves after dormancy is often supported by transfer of nutrients from old leaves as storage tissues in evergreen species (Millard and Grelet 2010). Second, total P concentration declined with leaf age because of the decline of certain P fractions. Higher inorganic P concentration in younger leaves is necessary for active synthesis and growth (Hom and Oechel 1983; Kitajima et al. 2002). Higher sugar P concentration in younger leaves is allocated to expand leaves and non-hardened tissues for frost tolerance (Chapin and Kedrowski 1983; Lambers et al. 2015b). Previous study shows that from young to mature leaves, the sugar P declined three- to five-fold and was replaced by non-phospholipids (i.e., galactolipids and sulfolipids) (Lambers et al. 2012). Expanding leaves, when compared with older leaves, may need to increased P investment in chloroplast membranes. Higher sugar P concentration may increase water mobility through membranes and prevent intracellular freezing and cell death (Chapin and Kedrowski 1983). These are the reasons why young leaves have a higher sugar P concentration than old leaves. Likewise, higher nucleic P concentration in younger leaves is required to meet the protein synthesis demands (Elser et al. 1996). Previous study showed that the concentration of nucleic acid P was positively correlated with the rates of protein synthesis and the division of cells in leaves (Hidaka and Kitayama 2013). Thus, expanding leaves and higher growth rates of young leaves than old leaves should require a greater investment in ribosomal RNA to produce the proteins required for growth (Serner and Elser 2002).

Similarly, we found that the resorption efficiencies of total P and various P fractions, with the exception of residue P, were higher in the young leaves compared with old leaves but the resorption proficiency did not differ (Table 1; Table S3). The proportion of residue P is higher than other P fractions in old leaves and the

proportion of inorganic P is highest in young leaves in our study. Previous studies have shown that active growth of young leaves would result in a strong sink for nutrients in plants which may result in high resorption efficiency (Nambiar and Fife 1987; Yuan et al. 2017). These results suggested that senesced leaves maintained a similar level of P concentration regardless of leaf age, the higher concentrations of inorganic P, sugar P and nucleic P in the young leaves directly caused the higher P resorption efficiency. Furthermore, young needles with a greater portion of mobile P that can be re-translocated, whereas plant allocated a greater amount of P to phosphoproteins synthesis in older leaves, which would contribute to the decline of the total P resorption efficiency with leaf age (Chapin III and Moilanen 1991; Helmisaari 1992; Yuan et al. 2017). Therefore, young leaves tend to have higher P resorption efficiency than old leaves due to the fact that more P is allocated to inorganic P for inorganic process than residue P for phosphoprotein.

In conclusion, leaves generally accumulate more P than N in Chinese fir plantation following nutrient addition. Declines in foliar N/P ratios in needle due to the combined N and P addition indicate that the relief or absence of P limitation is mostly caused by increasing the inorganic P and sugar P fractions in leaves. Although the resorption efficiency of each P fraction did not respond to chronic nutrient addition, the resorption efficiency of inorganic P and sugar P fractions were in the range from 50% to 75%, which are definitely an important nutrient conservation mechanism. Nutrient addition increases soil P supply but has no significant effect on P resorption efficiency, so we conclude that P resorption was not likely regulated by soil P supply. Our results suggested that Chinese fir increased its P absorption rate to increase the accumulation of inorganic P and sugar P fractions in response to changes in soil nutrient availability. Furthermore, plants may adapt to change environment not only by regulating nutrient resorption but also by altering root nutrient acquisition. Thus, future research should examine the relationship between root morphology and P absorption in respond to nutrient addition.

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