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Mixed Broadleaved Tree Species Increases Soil Phosphorus Availability but Decreases the Coniferous Tree Nutrient Concentration in Subtropical China

Wen-Sheng Bu [1,2], Han-Jiao Gu [1,2], Can-can Zhang [1,2], Yang Zhang [1], Anand Narain Singh [3], Xiang-Min Fang [1,2], Jing Fan [1,2], Hui-Min Wang [4] and Fu-Sheng Chen [1,2,*,†]

[1] Jiangxi Provincial Key Laboratory of Silviculture, College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China; bws2007@163.com (W.-S.B.); guhanjiao@163.com (H.-J.G.); brightzcc@163.com (C.-c.Z.) zhangyang0558@163.com (Y.Z.); xmfang2013@126.com (X.-M.F.); fxh2022@163.com (J.F.)

[2] Jiulianshan National Observation and Research Station of Chinese Forest Ecosystem, 2011 Collaborative Innovation Center of Jiangxi Typical Trees Cultivation and Utilization, Jiangxi Agricultural University, Nanchang 330045, China

[3] Department of Botany, Panjab University, Chandigarh 160014, India; dranand1212@gmail.com

[4] Qianyanzhou Ecological Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China; wanghm@igsnrr.ac.cn

* Correspondence: chenfush@yahoo.com; Tel./Fax: +86-791-83813243

† Current address: No. 1101 Zhiminda Road, Economic & Technological Development Zone, Nanchang 330045, China.

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Abstract: Phosphorus (P) is a key limiting nutrient in subtropical forests and mixed forests with broadleaved species have been expected to stimulate P cycling, compared to pure conifer plantations. However, the mixture effect of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) and broadleaved species on rhizosphere soil and coniferous tree P dynamics is unclear. In our study, eight plots of a single species of a Chinese fir plantation (pure plantation, PP) and eight mixed plantations (mixed plantation, MP) with broadleaved tree species (*Michelia macclurei* Dandy in Hunan Province or *Schima superba* Gardn. et Champ. in Fujian Province) were selected in subtropical China. Six P fractions in the rhizosphere and bulk soils were analyzed by a modified Hedley P fractionation method. Phosphorus fractions and nitrogen (N) concentrations in different root orders, different age fresh needles and twigs, and needle and twig litter of Chinese fir were measured. Our results showed that available P, slowly released P, occluded P, and the total extractable P in rhizosphere soil were significantly higher in MP than PP ($p < 0.05$). In contrast, P and N concentrations in the transportive roots and two-year old needles were generally higher in PP than MP. Meanwhile, the slowly released P, occluded P, total extractable P, and residual P in rhizosphere soil were negatively correlated with P concentrations in young (absorptive and transportive roots, one- and two-year old needles) but not old tissues (storative roots, three-year old needles and litters). In conclusion, mixture may increase soil P availability through the rhizosphere effect, but can decrease P and N concentration of Chinese fir tissues by competition between Chinese fir and broadleaved species. Clearly, the mixture effect may differ in soil and plant nutrients, and this issue needs be taken into consideration when converting a pure conifer plantation into a mixed-species forest.

Keywords: *Cunninghamia lanceolata*; mixture effect; nutrient cycling; rhizosphere effect; species competition

1. Introduction

Forest plantations in China have an area of 69 million hectares, occupy 31.8% of the total area of Chinese forests, and play important social, economic, and environmental roles [1]. However, most of these plantations were planted as monoculture conifers, such as Chinese fir (*Cunninghamia lanceolate* (Lamb.) Hook.) and *Pinus massoniana* Lamb., which might lead to nutrient imbalance, soil degradation, and the reduction of ecosystem stability [2]. In Southern China, Chinese fir has been widely planted and provides excellent commercial woods with easy processing because it is a native, fast-growing woody species with a 20–25 year rotation period and more than 1000 years of management practice history [3]. The removal of harvested wood and accumulated litter could alter the biogeochemistry in Chinese fir plantations, which may result in the decline of forest productivity due to multiple rotations [4]. Therefore, converting Chinese fir monoculture into mixed with broadleaved trees has become a common trend of forest management, since the mixed plantations seemed to have many advantages over pure stands, such as higher rates of litter decomposition and maintaining soil nutrient cycling [5], i.e., there were hardly any, or even negative effects of mixture on ecosystem functions, such as tree biomass accumulation and useful wood yield [6].

Phosphorus (P) is a key limiting nutrient in terrestrial ecosystems especially in the tropics and subtropics, where P is limited in the highly weathered soil and is found in pools with low amount available for plant [7]. Moreover, soil P utilization percentage is only 10%–25% in subtropical China [8] in comparison to P-rich soil from other tropics of the world. Shenoy et al. found that suboptimal levels of P could decrease crop growth and resulted in 5%–15% yield losses [9]. Many Chinese fir plantations have been established in these P-deficient sites, therefore, the availability of soil P is one of the most important limiting factors causing a decline in forest productivity because Chinese fir has a high use efficiency of which P is required for its optimal growth [8]. As mentioned above, the mixed-species forests have many advantages over pure plantations, and can improve root distribution patterns, plant residue compositions, and soil microbial diversity [10]. Theoretically, mixed forest plantations with Chinese fir and broadleaved trees would stimulate P cycling by mobilizing soil fixed P and increasing plant uptake, compared to pure stands of Chinese fir. However, the effect of mixed species on soil–tree P cycling in the Chinese fir forest ecosystem is little studied [8] and future P scenario cannot be effectively forecasted with the increasing conversion from pure plantations to mixed forests.

The nutrient availability is usually a major constraint to tree growth in most terrestrial ecosystems, while trees take up most of the mineral nutrients through the rhizosphere. The rhizosphere is a vital region of ecosystem biogeochemistry and governs the nutrient availability through plant roots to stimulate soil microbial activity [11]. Nutrients in the rhizosphere can rapidly transform from unavailable to available forms through the interaction of root exudates and microorganisms [12]. Plants generally secrete 10%–30% root exudates into rhizosphere soil through the root system. In order to increase the carbon availability in rhizosphere, microorganisms utilized those root exudates as food and energy [13]. Therefore, the microorganism population density might be much larger in the rhizosphere than in bulk soil. Plants absorb most mineral nutrients with effective nutrient transformation and mineralization dominated by rich microorganisms in rhizosphere soil [11]. Unfortunately, the difference of nutrient transformation and availability between the rhizosphere and bulk soil is less understood especially in forest ecosystems [14]. The modified P functional fractionation method in acidic soils provides an effective tool to explore the P supply process in the rhizosphere soil of subtropical forests [15].

On the other hand, the effects of species interactions on ecological processes such as nutrient cycling and tree growth in mixed stands have raised significant interest in recent years [2,16]. However, little is known about the influence of mixture on tree internal nutrient cycling [17], for example, the nutrient concentrations in various components of fresh tissues found in trees and their litters [18]. This result suggests that there is a change in plant physiology based on what is around the plant, or is it simply a function of competition? The understanding of internal nutrient cycling is of great importance because it not only depends on site nutrient availability but also reflects the competition among the different tree species in the mixed forest [19]. By now, mixed-species plantations containing a leguminous species have shown the potential to improve nutrient cycling [20]. However, there is a lack of evidence to validate the potential of mixed-species plantations without leguminous species, particularly in the tropics and subtopics [16]. There is no significant difference between mixtures and monocultures, when all of the empirical studies are combined [21]. The lack of a general trend, and the limited number of studies on the impact of mixed stands on tree nutrients, switches further studies. In addition, nutrient dynamics generally vary with the age of plant tissue. For example, more nutrients were resorbed to young leaves from old leaves when the acquisition of nutrients from the soil became more difficult [22]. Thus, the differentiation among the different ages of plant tissues might be helpful to explore nutrient internal cycles in response to the changing environment.

In the present study, eight plots of single-species Chinese fir plantation (pure plantation, PP) and eight plots of mixed plantation with a broadleaved tree species (mixed plantation, MP) with similar standing density in mature forest were selected to assess the effect of mixed species on soil and tree P cycling including belowground rhizosphere processes and nutrient concentrations of different functional root orders, and nutrient concentrations in aboveground components (fresh twigs, needles, and their litters) of different ages in Hunan and Fujian provinces of China. Both are the center distribution zones of Chinese fir production in China [23]. Our main hypotheses are: (1) the mixture with broadleaved trees improves soil P availability, and then increase tree nutrient levels; (2) the mixture effect (the difference between PP and MP) on rhizosphere soil P supply varies with P fraction, and depends on its rhizosphere effect (the increasing percentage in rhizosphere soil compared to bulk soils); and (3) the response of Chinese fir nutrients to the mixed with broadleaved tree varies with root orders, needle and twig ages due to combined effects of soil nutrient supplies, tree species competition, and plant physiological requirement. In addition, we explored the relationships among the nutrients of belowground component (root–soil), and aboveground nutrients (fresh twigs, needles, and their litters) in these ecosystems. Our results will help assess the potential effect of stand transformation from the monoculture plantation to mixed forest, and will provide theoretical support for forest management.

2. Materials and Methods

2.1. Study Sites

This study was conducted at two selected sites in subtropical China. One is located at Huitong Forest Ecological Station in Hunan Province (26°52′N, 109°42′E), and the other at Datian Experimental Forest Farm in Fujian Province (25°45′N, 117°33′E) (Figure S1). Both sites are approximately 1000 km away from each other but have a similar humid, mid-subtropical, monsoonal climate with the average annual temperatures of 15~18 °C and average annual precipitation of 1350~1450 mm. The zonal vegetation is evergreen broadleaved forest; but establishing forests by plantation is more pronounced, and this has occupied more than 90% due to logging. The altitudes of both sites are 500–600 m ASL, soil types belong to red soil, but are classified as Oxisol in Hunan Province and Humic Planosol in Fujian Province, respectively, according to the FAO classification system. Chinese fir has become the most important afforestation tree due to its fast-growing, easy processing, and wide utilization in both provinces [24].

2.2. Experimental Design and Investigation

At each selected site from both provinces, we randomly established total eight plots (circles with 200-m^2 size); At each site of both provinces, there are four plots in pure Chinese fir plantations and four plots in mixed plantations with a broadleaved tree species (*Michelia macclurei* in Hunan Province or *Schima superba* in Fujian Province), respectively, which are located on the mid-slope with the slopes ranging from 25° to 30°. Both *M. macclurei* and *S. superba* are native evergreen tree species, and widely afforested as pure or mixed forest due to rapid growth characteristic. In Hunan Province, both stand types were established in 1983 and common management practices were used in the early stages of the two stand types, including weeding and thinning. In Fujian Province, both stands were established in 1991 and similarly managed, such as weed-controlling during the first three years and thinning during the first 10 years (for more details see [23]). All sixteen selected plots were independently distributed in different hills, and generally far away, with more than 500 m between two plots of the same stand type.

In May 2013, the diameter at breast-height (DBH) and species of trees with a DBH > 2 cm were measured and recorded in all plots of both sites. The stand basal area was obtained at plot level. Meanwhile, general stand characteristics, including stem density, shrub density, and herb cover were investigated (See Table 1).

Table 1. Stand characteristics (means and standard errors, $n = 4$) of pure Chinese fir plantations (PP) and its mixed plantations with a broadleaved tree species (MP) at two sites located in Hunan and Fujian provinces of China.

Parameters	**Hunan**		**Fujian**	
	PP	**MP**	**PP**	**MP**
Basal area (m^2 ha^{-1})				
Chinese fir	35.31 ± 2.20	23.47 ± 2.26	69.75 ± 4.16	42.43 ± 2.02
Broadleaved tree	—	11.04 ± 1.21	—	11.97 ± 1.49
Total	35.31 ± 2.20	34.51 ± 2.23	69.75 ± 4.16	54.40 ± 2.96
Stem density (trees ha^{-1})				
Chinese fir	1410 ± 102	620 ± 86	2510 ± 129	2010 ± 76
Broadleaved tree	—	730 ± 68	—	710 ± 29
Total	1410 ± 102	1350 ± 47	2510 ± 129	2720 ± 85
Shrub density (stems ha^{-1})	8160 ± 1063	6320 ± 1038	14000 ± 2449	8500 ± 1000
Herb cover (%)	27.4 ± 12.4	14.1 ± 4.1	8.6 ± 4.0	2.6 ± 2.6
Understory plant biomass (g m^{-2})	154 ± 79	145 ± 42	214 ± 76	157 ± 63

Note: The broadleaved tree species are *Michelia macclurei* and *Schima superba* in Hunan and Fujian provinces, respectively.

2.3. Sampling

Within each circular plot in both PP and MP, three individuals of Chinese fir tree were chosen as reference plants and the rhizosphere and bulk soils, twig and needle litters under these tree canopies, roots of various orders, stems, fresh twigs, and needles varying with age in the trees were sampled. Rhizosphere soils were sampled using a hand shaking method and defined as the soils <4 mm away from the fine roots distributed 0–20 cm in the surface soil layer (>50% of the total Chinese fir fine roots [23]). Bulk soil was collected using soil cores (10 cm diameter) at 0–20 cm depth in the middle locations of forest gaps. Roots (living roots with <4 mm diameter) were extracted by shovel, the Chinese fir roots carefully collected by hand, and divided as three functional orders of absorptive roots (AR, the first three orders), transportive roots (TR, the 4th–5th orders), and storative roots (SR, >5th orders) in the laboratory [25]. Stems were sampled at breast height of the reference trees using an increment borer. Fresh needles and twigs from one first-order branch were collected from all three reference trees. Needles and twigs were divided into the first, second, and third orders of branching based on their ages (one-year old, two-year old, and three-year old needles or twigs) (see [18]). Meanwhile, twig and needle litters were directly cut from the trees using the combined method of people climbing and a tree pruner, since part of new branch litters (including the twig and needle litters) generally remain on the Chinese fir trunks. In our study, the concentrations of nutrients remaining in twig and needle litters were defined as nutrient resorption proficiencies in twig and needle organs, respectively [26]. All same samples were mixed as a sample within a plot. Soil and plant samples were air-dried at room temperature and dried at 60 °C for more than 72 h in an oven before nutrient measurement.

2.4. Chemical Analyses

Rhizosphere and bulk soils were cleared of roots and all organic debris, and ground to pass through a 0.25-mm sieve before analyses of P fractions, total P, organic carbon (C), and total N. In this study, total P concentrations of soil were determined by the molybdenum-antimony colorimetric method after samples through digestion with 1.84 M H_2SO_4 [27]. For the determination of extractable P, we employed improved Hedely P fractionation methods to quantify soil P functional fractions [28,29]. Air-dried soil samples were processed to follow the soil P fractionation sequential procedure [15]. The corresponding supernatants sequentially exacted with anion exchange resin (weak base), 0.5 M $NaHCO_3$, 0.1 M NaOH, 0.1 M NaOH with sonication, 1.0 M HCl were collected by centrifuge at 1.7×10^4 $m{\cdot}s^{-1}$ (3200 rpm) for five minutes, followed by filtering samples through a 0.45-µm micropore filter. Phosphorus concentration in each supernatant was determined by the phosphomolybdic acid blue color method. The extractable P, including Resin-P, $NaHCO_3$-P, NaOH-P, sonication-P, and HCl-P, are defined as available P, soluble P, slowly released P, occluded P, and weathered mineral P, respectively, based on their functions in soils [15]. The residual P is the difference between total P and extractable P.

Soil pH was determined using a pH Meter with a soil:water ratio of 1:2.5. Soil organic carbon (SOC) was determined by dichromate oxidation and titration with ferrous ammonium sulfate [29]. Total N was measured using the microkjeldahl method after digestion with 1.84 M H_2SO_4. Plant samples were washed with dematerialized water to remove dust, oven-dried, and ground and screened with a 0.25 mm sieve. Total N and total P concentrations in plant tissues were determined using the micro-Kjeldahl method and molybdenum-antimony colorimetric method, respectively [29].

2.5. Statistical Analyses

The data were tested for homogeneity of variances (Brown and Forsythe's variation of Levene's test) before statistical analysis. In order to assess the Chinese fir fine root function on soil P supply, the rhizosphere effect (%) of P availability was defined as the difference between rhizosphere and bulk soils. Multiple-way analysis of variance (ANOVA) was used to determine the effect of stand type, site location, soil sources (rhizosphere vs. bulk soil), or tissue ages, and the interactions between stand type and site location on nutrient variables. One-way ANOVA and least significant difference (LSD) was used to compare the differences of mean values among the three orders of roots, or three age classes of needles and twigs. Pearson's tests were used for comparing the significance of correlations among soil P fractions and tree nutrient concentrations. SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) [30] was used to perform all analyses. The standard 0.05 level was used throughout as a cutoff for statistical significance.

3. Results

3.1. Soil General Properties

Organic C and total N concentrations in rhizosphere and bulk soils were generally higher in MP than PP in both sites. However, the C/N ratio of rhizosphere soil in PP at the Hunan site was higher than that in MP, while it showed the opposite trend at the Fujian site. The C/N ratio in bulk soil was not significantly different between PP and MP in both sites. Organic C and total N were generally higher in rhizosphere than bulk soil under all the treatments. Meanwhile, organic C and C/N in rhizosphere and bulk soils were generally higher in the Fujian than in the Hunan sites, but the total N was not significantly different between both sites. In addition, there is not a significant difference in pH between rhizosphere and bulk soils and among the four treatments (Table 2).

3.2. Phosphorus Fractions in Rhizosphere and Bulk Soils

Average concentrations of soil available P, slowly released P, and occluded P (not soluble P), mineral P and residual P reflected significantly higher concentrations in MP than PP whether in rhizosphere or bulk soil (Figure 1 and Table S1). Average concentrations of soil soluble P, slowly released P, and residual P were higher in rhizosphere than bulk soil, and these three P fractions showed positive rhizosphere effects in all treatments. In contrast, the rhizosphere effects of available P, occluded P, and mineral P varied with forest type and site location (Figure 1 and Table S1). Soil P fractions including slowly released P, occluded P, mineral P, and residual P, but not available P, and soluble P was generally higher in the Fujian than the Hunan sites (Figure 1 and Table S1).

Table 2. General chemical properties of pure Chinese fir plantations (PP) and mixed plantations with a broadleaved tree species (MP) in Hunan and Fujian provinces of China. Values are means and standard errors of four replicates).

Parameter	**Hunan**		**Fujian**	
	PP	**MP**	**PP**	**MP**
pH				
Rhizosphere soil	4.36 ± 0.20 a	4.39 ± 0.09 a	4.35 ± 0.03 a	4.29 ± 0.03 a
Bulk soil	4.36 ± 0.21 a	4.32 ± 0.08 a	4.33 ± 0.01 a	4.20 ± 0.03 a
Organic carbon (g kg^{-1})				
Rhizosphere soil	16.0 ± 0.1 c	18.1 ± 0.8b c	21.7 ± 1.1 b	34.4 ± 2.7 a
Bulk soil	11.3 ± 2.0 d	15.6 ± 1.6 c	18.9 ± 2.0 b	23.3 ± 1.1 a
Total nitrogen (g kg^{-1})				
Rhizosphere soil	0.6 ± 0.0 b	1.0 ± 0.0 a	0.8 ± 0.1 ab	1.0 ± 0.1 a
Bulk soil	0.5 ± 0.00 b	0.8 ± 0.1 a	0.6 ± 0.1b	0.7 ± 0.1 ab
C/N				
Rhizosphere soil	27.6 ± 1.0 c	18.0 ± 0.9 d	30.7 ± 5.3 b	36.0 ± 1.2 a
Bulk soil	19.1 ± 1.5 b	20.9 ± 2.6 b	33.7 ± 2.0 a	35.3 ± 4.4 a

Note: Different letters indicate significant differences among four treatments at probability level of $p < 0.05$.

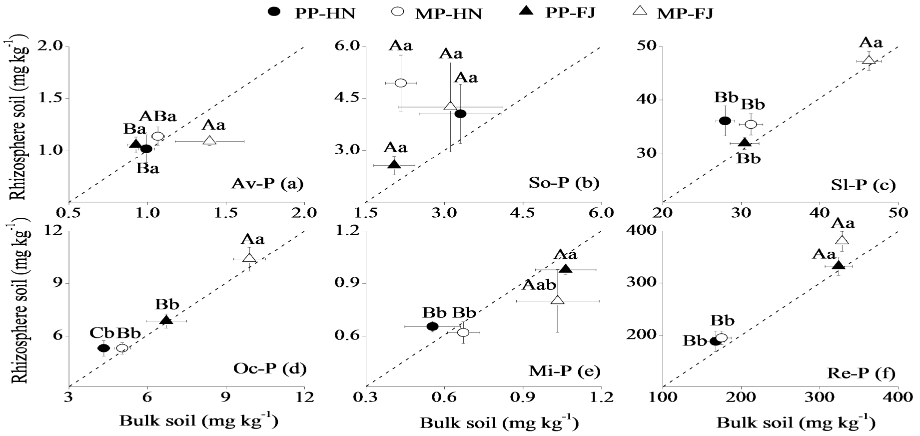

Figure 1. Variations in rhizosphere and bulk soil phosphorus fractions in pure Chinese fir plantations (PP) and mixed plantations with broadleaved tree species (MP) in Hunan and Fujian provinces of China. Note: The bar represents standard error and meaning of abbreviated words are as follow: PP-HN indicates the pure plantation in Hunan province, MP-HN indicates the mixed plantation in Hunan province, PP-FJ indicates the pure plantation in Fujian province, MP-FJ indicates the mixed plantation in Fujian province. Av-P indicates available P, So-P indicates soluble P, Sl-P indicates slowly released P, Oc-P indicates occluded P, Mi-P indicates mineral P, Re-P indicates residual P. The symbols located above and below the dotted line indicate the positive and negative rhizosphere effect, respectively. Among treatments, different small and capital letters indicate the significant differences in rhizosphere and bulk soils, respectively, probability level $p < 0.05$.

When the data from Hunan and Fujian were pooled, total P values were not significantly different between PP and MP either in rhizosphere or bulk soil, but the total extractable P was higher in MP than PP in both rhizosphere and bulk soils (Figure 2). The percentage of total extractable P to total P increased from 14.8% to 16.3% in bulk soil and from 13.8% to 16.8% in rhizosphere soil with the broadleaved tree species mixture. Among the five extractable fractions, slowly released P was the dominant form and its percentage to total extractable P ranged from 73.6% to 77.7%, followed by occluded P (11.2%~14.1%) in our study (Figure 2).

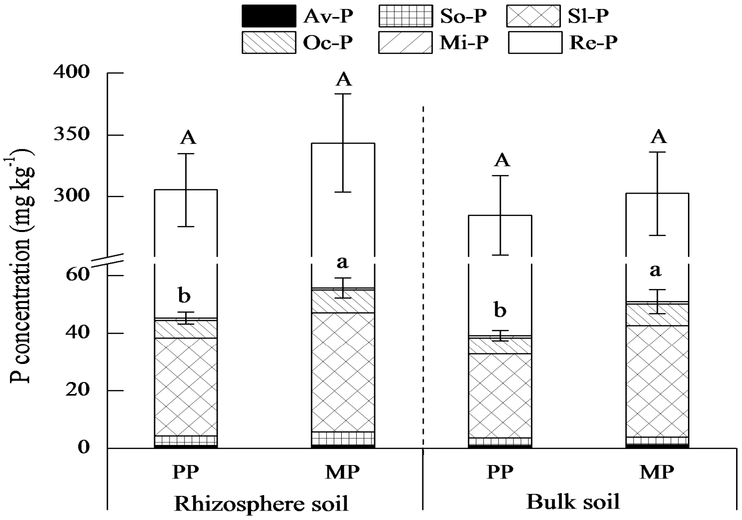

Figure 2. The components of soil phosphorus fractions in pure Chinese fir plantations (PP) and mixed plantations with broadleaved tree species (MP) in Hunan and Fujian provinces of China. Note: The bar represents standard error. Av-P indicates available P, So-P indicates soluble P, Sl-P indicates slowly released P, Oc-P indicates occluded P, Mi-P indicates mineral P, Re-P indicates residual P. Different small and capital letters indicate the significant differences of total extractable P (the sum of Av-P, So-P, Sl-P, Oc-P, and Mi-P) and total P, respectively, among the four soil sources (two forest types in rhizosphere or bulk soils, probability level $p < 0.05$.

3.3. Nutrient Levels in Different Root Orders

Total P concentration in absorptive and transportive roots is higher in PP than MP in the Fujian site, whereas it was not significantly different in roots between PP and MP in the Hunan site. Total N concentrations in absorptive and transportive roots were higher in PP than MP in each site of Hunan and Fujian provinces. Thus, the average N concentration in roots was also higher in PP than MP, although there were not statistical differences between both forest types in storative roots. The N/P ratio in all orders of roots seemed to be higher in PP than MP in the Hunan site (Figure 3 and Table S2). Meanwhile, total P and N concentrations were generally higher in absorptive than transportive and storative roots, and the N/P ratio was highest in storative roots, followed by transportive roots, and lowest in absorptive roots. In addition, the average P concentration was significantly higher in the Fujian than the Hunan site, while the average N concentration and N/P ratio showed the opposite trends (Figure 3).

3.4. Nutrient Distributions in Aboveground Tissues

Average P concentrations in the two-year old fresh needles were generally higher in PP than MP, although P concentrations in other aged needles were not significantly different between the two forest types (Figure 4a,b). In contrast, average N concentrations of all ages' needles and in each ages' needles were not significantly different between two forest types except higher N concentration in PP than MP in the two-year old fresh needles (Figure 4a,b and Table S3). Average P concentration in needles across all plots was highest in fresh needle of one-year old, followed by two- and three-year old needles and lowest in needle litter, while average N concentration was higher in fresh needles of one- and two-year old than three-year old needles and needle litter (Figure 4a,b). The ratio of N/P varied slightly between the two forest types and between the two site locations, but was higher in needle litter than fresh needles of various ages (Figure 4c).

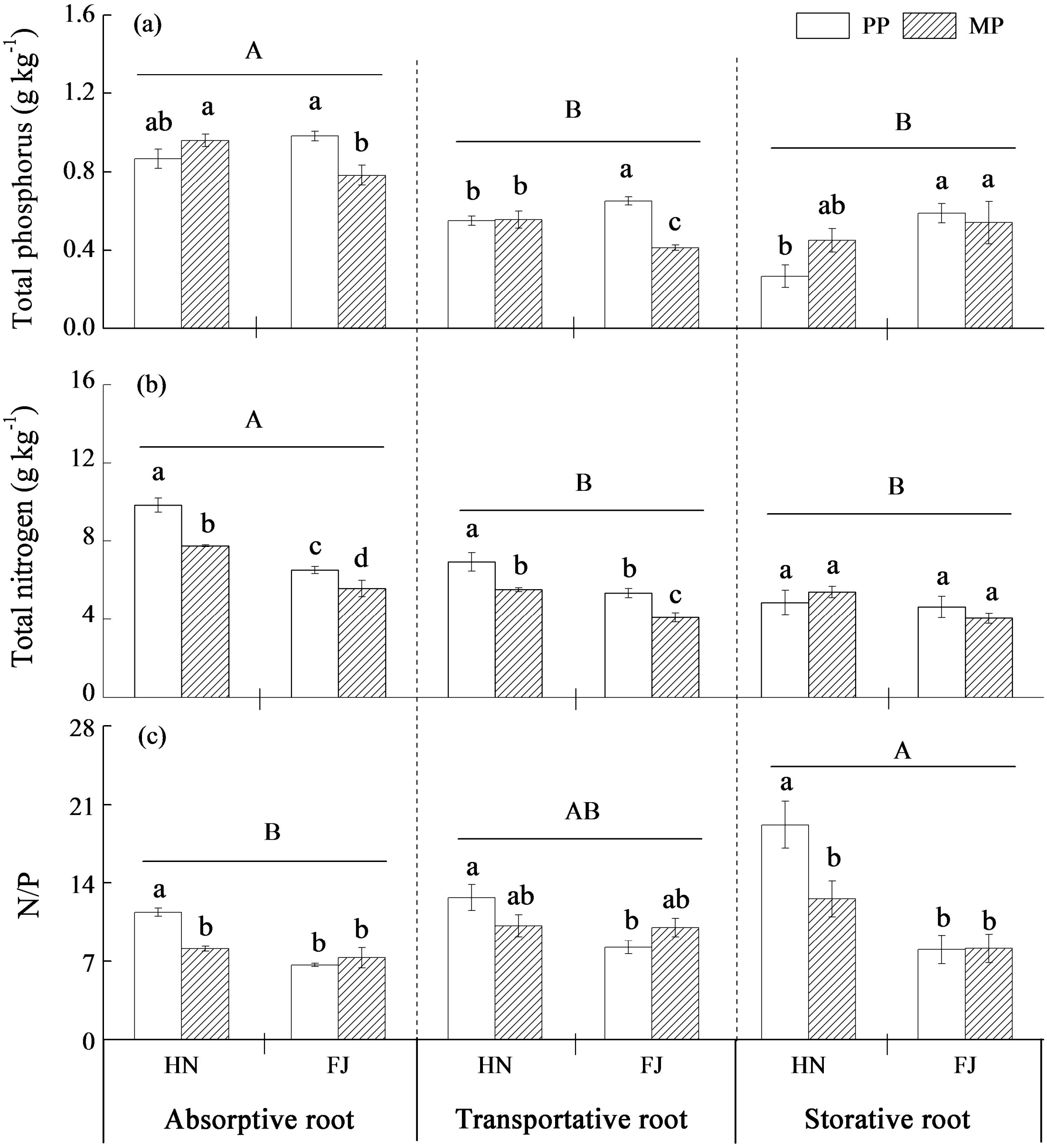

Figure 3. Phosphorus and nitrogen concentrations and N/P ratios of Chinese fir various functional roots in pure Chinese fir plantations (PP) and mixed plantations with broadleaved tree species (MP) in Hunan and Fujian provinces of China. (**a**) Total Phosphorus (g kg^{-1}), (**b**) Total nitrogen (g kg^{-1}), (**c**) N/P. Note: The bar represents standard error. Different small and capital letters indicate the significant differences among the four treatments within the root order, and among the three root orders, respectively, probability level $p < 0.05$.

Both P and N concentrations of twig were not significantly different between PP and MP, although N concentrations of the two- and three-year old twigs were significantly larger in PP than those in MP at the Hunan site. Additionally, those nutrient concentrations were highest in fresh twigs of one-year old, and lowest in twig litters, and significantly higher in Hunan than Fujian sites when just considered the effect of site location (Figure 5a,b and Table S4). The average N/P ratio was not significantly different between two forest types and between two site locations, but highest in twig litters and lowest in fresh twig of one-year old (Figure 5c and Table S4). In addition, the stem N and P concentrations and N/P ratio were not significantly different between both forest types except for a higher N/P ratio in PP than MP in the Fujian site (Figure 5).

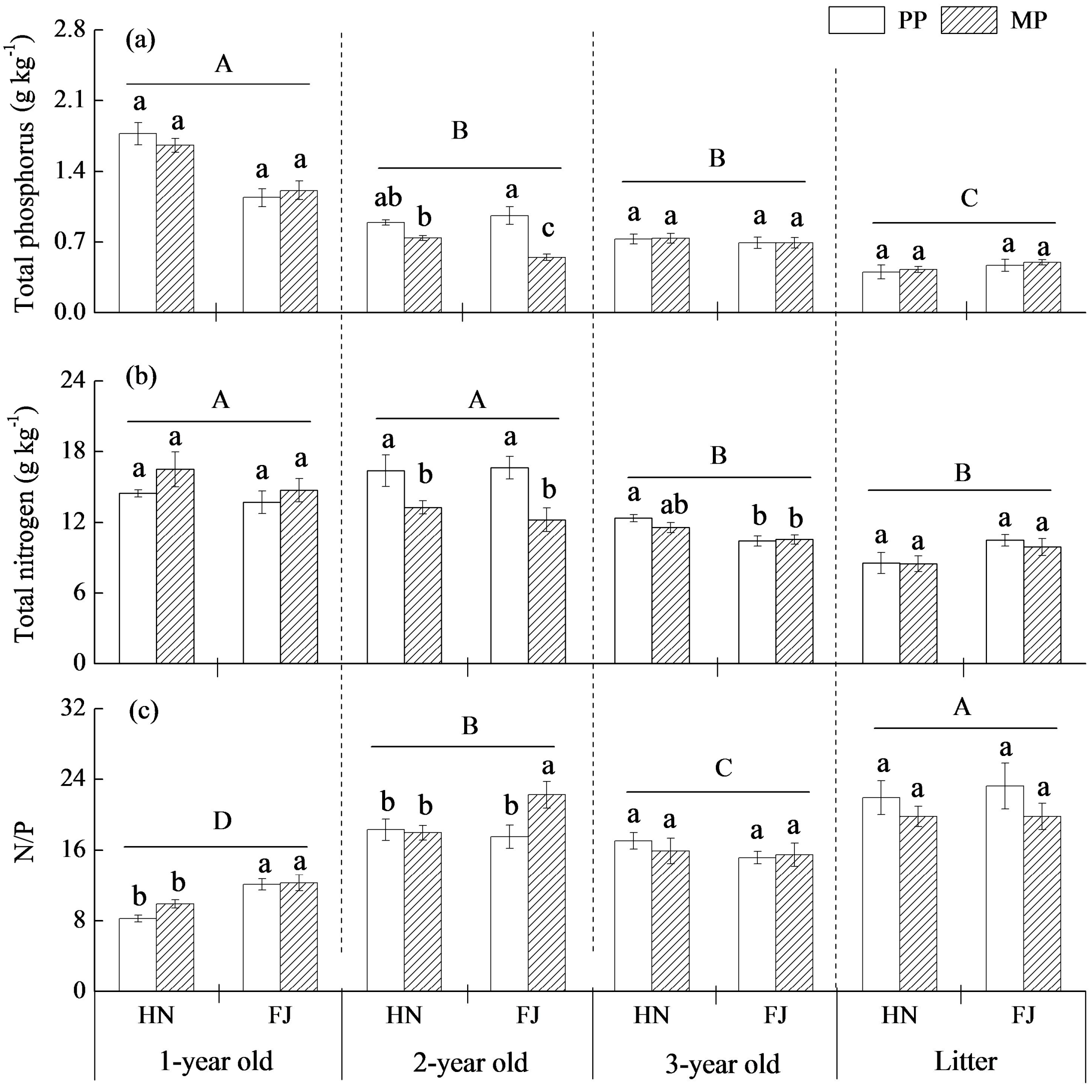

Figure 4. Phosphorus and nitrogen concentrations and N/P ratios of Chinese fir different-aged fresh needles and needle litters between pure Chinese fir plantations (PP) and mixed plantations with broadleaved tree species (MP) in Hunan and Fujian provinces of China. (**a**) Total Phosphorus (g kg^{-1}), (**b**) Total nitrogen (g kg^{-1}), (**c**) N/P. Note: The bar represents standard error. Different small and capital letters indicate the significant differences among the four treatments within a same age needle, and among these four age needles, respectively, probability level $p < 0.05$.

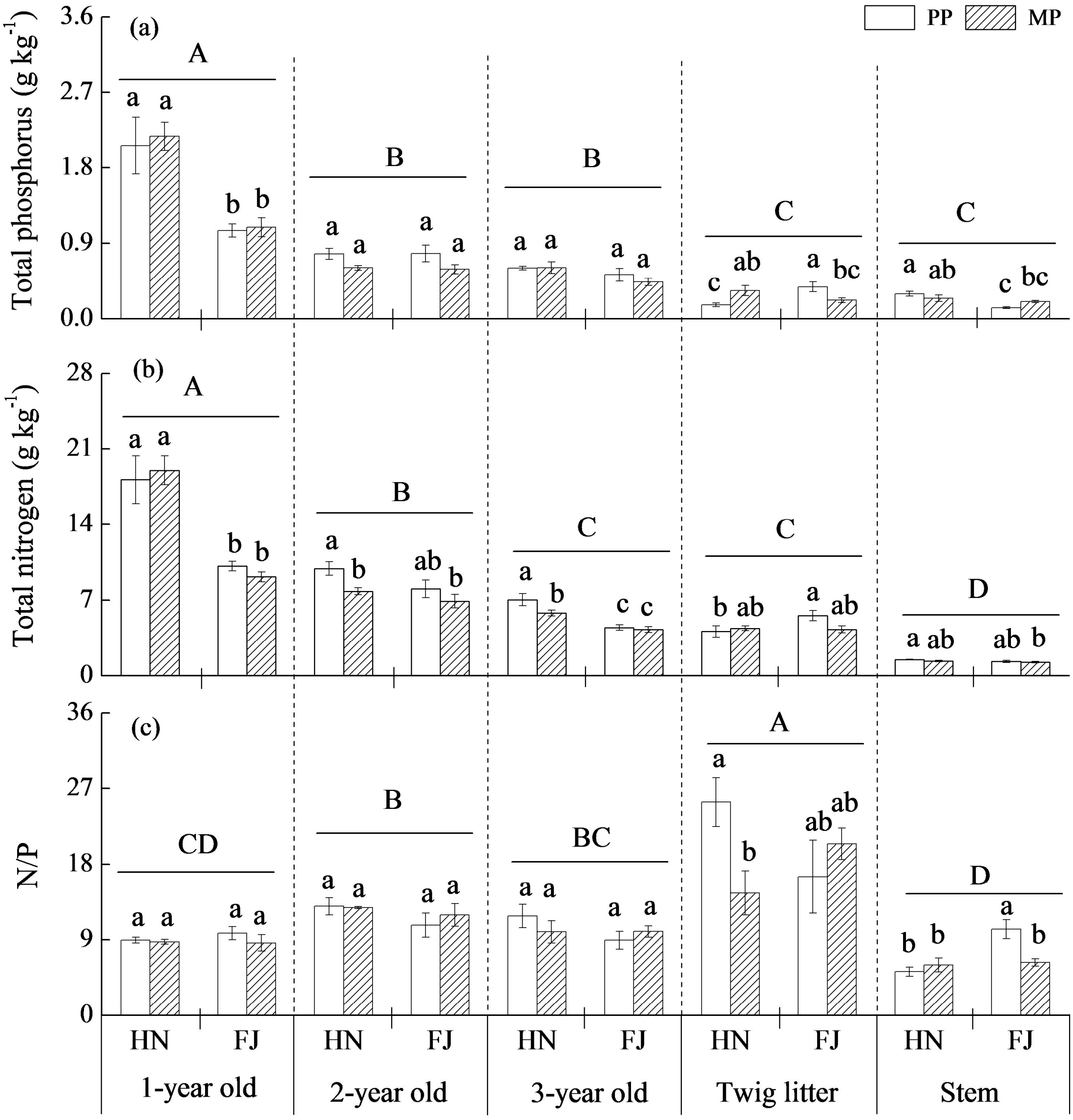

Figure 5. Phosphorus and nitrogen concentrations and N/P ratio of Chinese fir different-aged fresh twigs, twig litters, and stems between pure Chinese fir plantations (PP) and mixed plantations with broadleaved tree species (MP) in Hunan and Fujian provinces of China. (**a**) Total Phosphorus (g kg^{-1}), (**b**) Total nitrogen (g kg^{-1}), (**c**) N/P. Note: The bar represents standard error. Different small and capital letters indicate the significant differences among the four treatments within a same-aged twig, and among the four ages of twigs and stems, respectively, probability level $p < 0.05$.

3.5. Linkages Among Soil, Root and Needle Nutrients

Unlike our expectations, we did not find any positive relationships between all P fractions in rhizosphere soils and P concentrations in roots of different functions and needles of various ages. In contrast, slowly released P, occluded P, and residual P negatively correlated with the P concentrations in some functional roots and fresh needles of some ages, and the extractable P negatively correlated with P concentration in one-year old needles (Table 3). Meanwhile, the rhizosphere effect of some P fractions negatively correlated with P concentration in some roots, fresh needles, and needle litters except for a positive correlation between soluble P and P concentration in absorptive roots (Table 3).

Likewise, slowly released P, occluded P, mineral P, extractable P, and residual P in rhizosphere soils negatively correlated with N concentrations in transportive or storative roots, and in two- or three-year old needles. However, rhizosphere soil soluble P concentration was positively correlated with absorptive root N concentration, and both mineral P and extractable P were positively correlated with N concentration in one-year old needle (Table 4). Meanwhile, the rhizosphere effects of slowly released P and residual P were positively correlated with N concentrations in two- and three-year old needles, but negatively correlated with N concentration in one-year old needle. In addition, N concentration in needle litter was not significantly correlated with any P fractions and their rhizosphere effects (Table 4).

Table 3. Correlation efficiencies (n = 16) between rhizosphere soil phosphorus supplies (rhizosphere soil P fractions and their rhizosphere effects) and tree tissue phosphorus concentrations (total P of various Chinese fir tissues) under all studied stands in Hunan and Fujian provinces, China.

Variables	**Total P of Roots**			**Total P of Fresh Needles**			**Total P of Needle Litter**
	Absorp-tive	**Transpor-tive**	**Storative**	**1-yr old**	**2-yr old**	**3-yr old**	
P fractions in rhizosphere soil							
Av-P	−0.04 ^{ns}	−0.16 ^{ns}	−0.11 ^{ns}	−0.34 ^{ns}	−0.12 ^{ns}	0.05 ^{ns}	−0.21 ^{ns}
So-P	0.08 ^{ns}	−0.26 ^{ns}	−0.50 ^{ns}	0.19 ^{ns}	−0.26 ^{ns}	−0.16 ^{ns}	−0.35 ^{ns}
Sl-P	**−0.58** *	**−0.77** **	−0.11 ^{ns}	−0.21 ^{ns}	**−0.78** **	−0.02 ^{ns}	−0.04 ^{ns}
Oc-P	−0.39 ^{ns}	**−0.63** **	0.25 ^{ns}	**−0.64** **	**−0.62***	−0.10 ^{ns}	0.21 ^{ns}
Mi-P	0.34 ^{ns}	0.25 ^{ns}	0.25 ^{ns}	−0.38 ^{ns}	0.16 ^{ns}	0.17 ^{ns}	0.24 ^{ns}
Ex-P	−0.18 ^{ns}	−0.31 ^{ns}	0.47 ^{ns}	**−0.86** **	−0.40 ^{ns}	−0.06 ^{ns}	0.39 ^{ns}
Re-P	−0.49 ^{ns}	**−0.76** **	−0.11 ^{ns}	−0.29 ^{ns}	**−0.76** **	−0.06 ^{ns}	−0.04 ^{ns}
Rhizosphere effect of phosphorus fractions							
Av-P	0.43 ^{ns}	0.36 ^{ns}	−0.27 ^{ns}	−0.12 ^{ns}	0.43 ^{ns}	0.21 ^{ns}	−0.34 ^{ns}
So-P	**0.51** *	0.00 ^{ns}	−0.17 ^{ns}	0.08 ^{ns}	−0.08 ^{ns}	0.10 ^{ns}	−0.27 ^{ns}
Sl-P	−0.02 ^{ns}	0.02 ^{ns}	**−0.51** *	0.42 ^{ns}	0.21 ^{ns}	−0.03 ^{ns}	**−0.60** *
Oc-P	0.07 ^{ns}	−0.12 ^{ns}	−0.39 ^{ns}	−0.04 ^{ns}	0.05 ^{ns}	−0.18 ^{ns}	**−0.54** *
Mi-P	−0.02 ^{ns}	0.27 ^{ns}	0.01 ^{ns}	0.48 ^{ns}	0.35 ^{ns}	−0.08 ^{ns}	0.03 ^{ns}
Ex-P	0.15 ^{ns}	**−0.5 2** *	−0.31 ^{ns}	0.06 ^{ns}	−0.39 ^{ns}	0.38 ^{ns}	−0.03 ^{ns}
Re-P	0.18 ^{ns}	0.03 ^{ns}	**−0.50** *	0.34 ^{ns}	0.19 ^{ns}	−0.06 ^{ns}	**−0.69** **

Note: Av-P indicates available P, So-P indicates soluble P, Sl-P indicates slowly released P, Oc-P indicates occluded P, Mi-P indicates mineral P, Re-P indicates residual P. ns indicates not significant, * $p < 0.05$, ** $p < 0.01$.

Table 4. Correlation efficiencies (n = 16) between rhizosphere soil phosphorus supplies (rhizosphere soil P fractions and their rhizosphere effects) and tree tissue nitrogen concentrations (total N of various Chinese fir tissues) under all studied stands in Hunan and Fujian provinces of China.

Variables	**Total N of Roots**			**Total N of Fresh Needles**			**Total N of Needle Litter**
	Absorp-tive	**Transpor-tive**	**Storative**	**one-year old**	**two-year old**	**3-year old**	
Phosphorus fractions in rhizosphere soil							
Av−P	−0.18 ^{ns}	−0.31 ^{ns}	−0.14 ^{ns}	−0.24 ^{ns}	−0.09 ^{ns}	−0.12 ^{ns}	−0.14 ^{ns}
So−P	**0.51** *	−0.33 ^{ns}	0.03 ^{ns}	−0.49 ^{ns}	0.11 ^{ns}	−0.10 ^{ns}	0.01 ^{ns}
Sl−P	−0.05 ^{ns}	**−0.55** *	−0.13 ^{ns}	−0.02 ^{ns}	−0.38 ^{ns}	−0.46 ^{ns}	−0.32 ^{ns}
Oc−P	−0.11 ^{ns}	−0.46 ^{ns}	**−0.60** *	0.34 ^{ns}	**−0.71** **	**−0.73** **	−0.39 ^{ns}
Mi−P	−0.07 ^{ns}	0.16 ^{ns}	−0.33 ^{ns}	**0.69** **	**−0.51** *	−0.28 ^{ns}	−0.33 ^{ns}
Ex−P	−0.31 ^{ns}	−0.36 ^{ns}	**−0.70** **	**0.50** *	**−0.76** **	**−0.72** **	−0.43 ^{ns}
Re−P	0.03 ^{ns}	**−0.57** *	−0.25 ^{ns}	−0.01 ^{ns}	−0.44 ^{ns}	**−0.54** *	−0.34 ^{ns}
Rhizosphere effect of phosphorus fractions							
Av−P	−0.12 ^{ns}	0.27 ^{ns}	0.11 ^{ns}	−0.12 ^{ns}	0.12 ^{ns}	0.17 ^{ns}	−0.14 ^{ns}
So−P	0.46 ^{ns}	−0.14 ^{ns}	−0.03 ^{ns}	−0.35 ^{ns}	−0.02 ^{ns}	−0.03 ^{ns}	0.11 ^{ns}
Sl−P	−0.09 ^{ns}	0.27 ^{ns}	**0.55** *	**−0.58***	**0.61** *	**0.72** **	0.24 ^{ns}
Oc−P	−0.06 ^{ns}	0.02 ^{ns}	−0.10 ^{ns}	−0.49 ^{ns}	0.31 ^{ns}	0.28 ^{ns}	0.01 ^{ns}
Mi−P	0.04 ^{ns}	0.47 ^{ns}	0.25 ^{ns}	0.07 ^{ns}	0.30 ^{ns}	**0.56** *	0.26 ^{ns}
Ex−P	0.38 ^{ns}	−0.47 ^{ns}	0.14 ^{ns}	−0.21 ^{ns}	0.12 ^{ns}	−0.24 ^{ns}	−0.28 ^{ns}
Re−P	0.05 ^{ns}	0.20 ^{ns}	0.40 ^{ns}	**−0.68** **	**0.54** *	**0.62** *	0.23 ^{ns}

Note: Av−P indicates available P, So−P indicates soluble P, Sl−P indicates slowly released P, Oc−P indicates occluded P, Mi−P indicates mineral P, Re−P indicates residual P. ns indicates not significant, * $p < 0.05$, ** $p < 0.01$.

4. Discussion

Overall, there is a trend of converting pure plantations into mixed plantations for increasing stability and sustainability due to, in theory, complementary resource use, environmental benefits, and soil improvement in mixed forests [5]. However, the practical effect does not always match with our expectation. Until now, there have been few reports on the negative effect of mixture with broadleaved trees and coniferous trees [31].

4.1. Mixture Effect on Nutrient Concentrations in Rhizosphere and Bulk Soils

Our results showed that organic C, total N, and total extractable P in both rhizosphere and bulk soils were higher in MP than PP. In other words, mixed Chinese fir plantations with broadleaved species increased nutrient concentrations in both rhizosphere and bulk soils. These results were in line with the hypothesis that the mixture with broadleaved trees improves soil P availability in Chinese fir plantations. Greater litter production or changes in the timing of litter inputs or differences of leaf nutrient concentration in mixed-species plantations could increase the soil nutrient supply, relative to monocultures, if decomposition rates were constant or faster [30]. Generally, the decomposition rate of litter and fine roots for coniferous species are slower than broadleaf species [32]. Additionally, some studies support that the decomposition rate of leaves and roost for *M. macclurei* and *S. superba* were faster than those of Chinese fir [33,34]. Moreover, compared with a pure Chinese fir plantation, the soil fertility and nutrient return were raised significantly in the mixed Chinese fir plantation with *M. macclurei*, due to substantial amount of litter and higher turnover rate of fine root [24]. Therefore, broadleaved trees with higher nutrient concentrations in the mixture could increase soil nutrient availability by altering the amount and quality of litter input as well as the amount and chemical composition of the root mass and exudates through fine root decomposition.

4.2. Mixture Effect Varies with Soil P Fraction

The average concentrations of soil available P, slowly released P, and occluded P, but not soluble P, mineral P, and residual P, were significantly higher in MP than PP and three of these showed positive rhizosphere effects. Since P is an element of depositional cycle, P absorption by plants depends on its concentration gradient and diffusivity in the soil near the roots. Both available P and soluble P are labile and are considered as the available fractions for plant growth [35]. Some studies have found that amending phosphates increases the immediate phosphorus availability and the rate of available P dissolution can be enhanced by the rhizosphere effect [36]. Moreover, the litter decomposition experiment of *S. superba* on a Chinese fir plantation shows that P concentration in the *S. superba* litter dropped rapidly, which could release 32% of the initial P into the soil during the first three months in Hunan Province [37].

Furthermore, the total extractable P was higher in MP than PP in both rhizosphere and bulk soils through the introduction of a broadleaf species in the mixed plantation. Recent studies indicated that the inactive P fractions could be converted into plant available forms with the help of the necessary manipulation of the rhizosphere environment [38]. The release of root exudates, such as organic ligands, is an activity of the root that can alter the concentration of P in the soil solution [12]. Some studies showed that cyclic dipeptides, which caused autoinhibition of Chinese fir, may be released into the soil through litter decomposition and root exudation. Moreover, root exudates provided more contributions to soil cyclic dipeptide levels than litter in Chinese fir plantations [39]. Thus, the introduction of broadleaf species to a pure Chinese fir plantation may relieve the autoinhibition of Chinese fir and alter the concentration of P in the soil solution. Furthermore, *S. superba* is a Mn-accumulating subtropical tree species and Mn hyperaccumulation is associated with Mn mobilization in the rhizosphere, most likely due to the release of protons. The carboxylates generated to produce the protons released into the rhizosphere are used internally in the plant to mobilize soil inorganic and organic P [40]. Therefore, the overall combination of changes in the P fractions in soil demonstrates that a mixture effect may induce an increase in soil P availability through the solubility of the unavailable form of soil P and the rhizosphere effect. After all, the mixture effect on P availability in rhizosphere soil varies with the P fraction, and may depend on the litter decomposition and root exudates of introduced broadleaf species.

4.3. The Response of Chinese Fir to Mixture Effect Varies with Root Orders, Needle, and Twig Ages

Our results showed that P and N concentrations in one-year old fresh needles and one-year old twigs were not significantly different between PP and MP in both study sites, although the mixture effect may induce an increase in soil P availability. Generally, evergreen trees tend to maintain a relatively favorable nutrient status in active young leaves for positive carbon (C) gain and high N use efficiency [41]. Trees can transport nutrients from old and senescing leaves to new organs to support new growth, which has been verified as a key mechanism of nutrient conservation and reuse in plants [42]. Thus, old leaves might be more sensitive to environmental variation, such as those mixed with a broadleaf species than young leaves. Generally, P and N concentrations in absorptive and transportive roots, two-year old fresh needles and two-year old twigs were significantly higher in PP than MP, although these concentrations in the other tissues were not different between both stand types. These results suggest that the introduction of a broadleaf species to pure Chinese fir plantation might decrease the nutrient concentration of Chinese fir. In contrast, most of studies suggested that mixed species stands have higher nutrient availability than monoculture, even in the absence of N-fixing species [43], though few studies have contrasted nutrient concentrations for species in mixtures [21]. Therefore, foliar nutrient concentrations did not show a general trend in the mixtures studied so far. Moreover, since the Chinese fir biomass in the mixed plantation was lower than that in the pure Chinese fir stand in our study, we deduced that lower P concentration in Chinese fir tissue may be attributing to the stronger competition with the broadleaved tree. Obviously, our result did not support the hypothesis that the mixture can increase tree tissue nutrient availability but, rather, supported the hypothesis that the response of Chinese fir nutrients to the mixed with broadleaved tree varies with root orders, and needle and twig ages. These results may be caused by the lower competition of Chinese fir than broadleaved species for nutrient uptake.

4.4. Linkages between Soil, Root, and Needle Nutrients

For the linkages among soil, root, and needle nutrients, we did not find any positive relationships between all P fractions in rhizosphere soil and P concentration in roots of different orders and in leaves of various ages. In contrast, slowly released P, occluded P, and residual P negatively correlated with the P concentrations in some root orders and fresh leaves of some ages, and the extractable P negatively correlated with P concentration in one-year old needle. Many studies suggest that any shift in plant species composition, which is able to alter soil nutrient stoichiometry, can influence rhizosphere microbial and soil enzyme activities, which can further induce plant community species shifts and alter ecosystem function [44]. Two adjacent plants can simultaneously participate in competition and facilitation processes, and the direction and intensity of plant interactions are determined by the sum of the co-occurring negative and positive effects of one to another. These negative linkages may cause by the negative plant–plant interactions between Chinese fir and the broadleaf species. In general, plants take up most mineral nutrients through the rhizosphere where microorganisms interact with root exudates. A study reported that the seedling survivorship of *S. superba* was significantly inhibited by *eucalyptus* robusta Smith litter addition alone, meanwhile the seedling height of *S. superba* and *M. macclurei* was significantly suppressed when eucalyptus roots were present [45]. This negative nutrient feedback (homeostasis) supports plant species coexistence with lower proportional changes in consumer stoichiometry compared with resource stoichiometry [46]. Evaluating homeostatic relationships can provide valuable insight into assessing plant competition or plant coexistence [47]. Therefore, negative relationships between P fractions in rhizosphere soil and tree tissue nutrients of Chinese fir may be caused by plant competition between Chinese fir and the broadleaved species.

5. Conclusions

We found that, compared with the Chinese fir plantation, the mixed Chinese fir plantation with a broadleaved species increased nutrient availability in both rhizosphere and bulk soils. Meanwhile, the introduction of broadleaf species to pure Chinese fir plantation generally decreased the tree nutrient availability of the transportive root and two-year old needles of Chinese fir, possibly due to the lower competition of Chinese fir than broadleaved species for nutrient uptake. Therefore, the effect of mixture may bring about a negative nutrient feedback between P availability in rhizosphere soil and P concentration in plant tissues attributed to root exudates and plant competition between Chinese fir and broadleaf species. In conclusion, replacing monoculture plantations of Chinese fir into mixed-species forest promotes soil nutrient availability, and it might be an option for multi-purpose forest management in China, from where the largest areas of Chinese fir plantations alter the functioning of the soil ecosystem. However, the mixed forest may affect P and N stocks of Chinese fir tissues. Thus, the mixture effect may differ in soil and plant nutrients, and when soil nutrient availability is expected to be improved, converting a pure conifer plantation into a mixed-species forest can be considered.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/4/461/s1, Figure S1: The study sites in Huitong Forest Ecological Station of Hunan Province and Datian Experimental Forest Farm of Fujian Province, Table S1: Summary of ANOVA about effects of forest type, soil type, site location and their interaction on phosphorus fractions under pure Chinese fir plantation (PP) and its mixed plantation with broadleaved tree species (MP) at Hunan and Fujian provinces, China, Table S2: ANOVA of effect of forest type, root functional order, site location and their interaction on root nitrogen, phosphorus concentrations and N/P between pure Chinese fir plantation (PP) and mixed plantation with broadleaved tree species (MP) at Hunan and Fujian province, China.. Table S3: ANOVA of effect of forest type, leaf age, site location and their interaction on leaf nitrogen, phosphorus concentrations and N/P ratio between pure Chinese fir plantation (PP) and mixed plantation with broadleaved tree species (MP) at Hunan and Fujian province, China. Table S4: ANOVA of effect of forest type, twig age, site location and their interaction on twig nitrogen, phosphorus concentrations and N/P ratio between pure Chinese fir plantation (PP) and mixed plantation with broadleaved tree species (MP) at Hunan and Fujian province, China.

Author Contributions: F.-S.C. contributed to the study conception and design. Material preparation, data collection and analysis were performed by W.-S.B., H.-J.G., C.-c.Z., Y.Z., A.N.S., X.-M.F., J.F., H.-M.W. and F.-S.C. The first draft of the manuscript was written by W.-S.B. and all authors commented on previous versions of the manuscript. All authors have read and agreed to the published version of the manuscript

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