Age-related C:N:P stoichiometry in two plantation forests in the Loess Plateau of China

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ABSTRACT

The nutrient stoichiometry in fine roots plays an important role in terrestrial carbon (C) and nutrient cycling, but how it changes with stand age in forests is poorly understood. In this study, we investigated fine root C, nitrogen (N), and phosphorus (P) concentrations and age sequence ratios (10-, 25-, and 40-year stands) of *Pinus tabulaeformis* Carr. (evergreen) and *Robinia pseudoacacia* Linn. (deciduous) forests on the Loess Plateau, China. Fine root C, N, and N:P of *R. pseudoacacia* were 22, 23, and 32% greater in 40-year stands than in 10-year stands, respectively, but did not differ significantly in *P. tabulaeformis* stands of different ages. Fine root P and C:P of both tree species significantly differed with respect to stand age. Fine root C:P and N:P of *R. pseudoacacia* were lower in 10- and 25-year stands than in 40-year stands. The age-related patterns of C, N, and C:N in fine roots were similar to those in green leaves. Fine root N:P of *R. pseudoacacia* was positively related to soil N:P. Our results suggest that stand age (temporal factor) plays a key role in fine root C:N:P stoichiometry in temperate forests. Differences in fine root C:N:P stoichiometry between *P. tabulaeformis* and *R. pseudoacacia* suggest that the nutrient-use strategy in evergreen tree forests is more conservative than in deciduous tree forests as age increases.

1. Introduction

The most physiologically active root zones are fine roots (≤ 2 mm in diameter). These sensitive organs respond to soil nutrient supply (Jackson et al., 1997; Liu et al., 2014). Nitrogen (N) and phosphorus (P) are the most important essential nutrients because they are used to synthesize a range of molecules with crucial structural and functional roles (Elser et al., 1996; Niklas and Cobb, 2005; Vrede et al., 2004). A key pathway of nutrient flux in terrestrial ecosystems is nutrient release from decomposing roots (Liu et al., 2010; Yuan and Chen, 2010b). Fine roots often equal or exceed decomposing foliage in their annual carbon (C) and nutrient inputs into the soil (Norby et al., 2000).

Ecological stoichiometry, the study of the balance of energy and chemical elements in biological systems, is a powerful tool for understanding biological processes from the level of individual organisms to that of the ecosystem (Elser, 2000). Plant C:N:P ratios are critical indicators of ecosystem processes (Elser et al., 2007; Güsewell, 2004; Jiao et al., 2013; Vitousek et al., 2010; Yuan and Chen, 2015). Plant physiologists have largely focused on plant N and P stoichiometry in green leaves (Han et al., 2005; He et al., 2008; Niklas et al., 2005). However, our knowledge of the C:N:P stoichiometry of fine roots is underdeveloped (Gordon and Jackson, 2000). Previous studies reported that fine root N:P declined exponentially with increasing latitudes (Yuan et al., 2011), which provided context for N:P stoichiometry on a large spatial scale. However, to our knowledge, the temporal variability of C:N:P stoichiometry in fine roots has not been investigated.

It is largely unknown whether fine root C:N:P stoichiometry varies with respect to stand age in forests. A recent study showed that in forests the C:N ratio in aboveground biomass increased significantly with stand age (Yang and Luo, 2011). Plant C:N (Yang and Luo, 2011), and fine root N:P (Yuan and Chen, 2010b) ratios significantly differed among different tree species. Thus, it is necessary to determine how the nutrient stoichiometry in different tree species changes with respect to stand age at local scales. Soil nutrient concentrations often change over

time in forests (Fan et al., 2015), and these changes directly influence nutrient uptake and elemental stoichiometry in plants.

The Loess Plateau of China has worldwide recognition for its severe soil erosion due to deforestation and other human activities. The "Grain for Green" project is a vegetation recovery program aimed at converting sloping cropland into woodland or grassland (Zhou et al., 2009). Afforestation has been a key measure to control the ecological problem in China's Loess Plateau during the past four decades (Jiao et al., 2012). *Pinus tabulaeformis* Carr. and *Robinia pseudoacacia* Linn. are often selected for afforestation in this region and are widely planted in arid and semiarid areas (Jin et al., 2011; Zhou and Shangguan, 2007). Vegetation restoration with these two plantation forests improves soil properties (Qiu et al., 2010), increases microbial activities (Yuan and Yue, 2012), and combats soil erosion (Zhang et al., 2010). Few studies have examined different age phases of fine roots during vegetation restoration (Chang et al., 2012; Chen et al., 2016), or measured age-related patterns of fine root C:N:P stoichiometry. To ensure the continued success of vegetation restoration in this arid/semiarid region, it is necessary to study fine root dynamics and nutrient acquisition during stand aging.

In this study, we investigated the fine root C:N:P stoichiometry with respect to stand age (10-, 25-, and 40-year stands) in *P. tabulaeformis* and *R. pseudoacacia* plantation forests on the Loess Plateau of China. We hypothesized that fine root C:N:P changes with stand age and that the age-related pattern would differ between tree species because of differences in the plants' lifestyle (Yang and Luo, 2011; Yuan and Chen, 2010b). We also hypothesized that fine roots and green leaves would have similar age-related patterns of C:N:P stoichiometry, because they are both metabolically active organs (Yuan et al., 2011).

2. Materials and methods

2.1. Study area

The study was conducted in the southern region of Yan'an City, Shaanxi Province, China (36°9′–36°29′ N, 109°16′–109°33′ E) (Fig. 1). This region has a semiarid continental climate with a mean annual temperature of 9 °C. The average annual precipitation is 560 mm, mostly occurring in July and August. The soil is described as Calcaric Cambisols based on the FAO classification system. *Pinus tabulaeformis* Carr. and *Robinia pseudoacacia* Linn. were planted in large areas between 1953 and 2003 to conserve soil and water. The tree coverage rate reached 60–70% in *P. tabulaeformis* stands, with minimal understory shrubs and grasses. The numbers and coverage of species in the understory vegetation of *R. pseudoacacia* stands were no more than ten species and 10–22% coverage, respectively.

2.2. Field sampling and laboratory analyses

Two tree species (*P. tabulaeformis* or *R. pseudoacacia*) and stand age classes (10-, 25-, and 40-year stands) were each replicated three times, for a total of eighteen sampling stands. All stands were converted from cropland and had similar slopes, which had the same history and land management prior to afforestation. The diameter at breast height (DBH) and tree height of each experimental stand are presented in Table 1.

Sampling of plants was conducted in July 2014 from one 10 m × 10 m plot within each sampling stand. We collected approximately 0.5 kg of green leaves from the upper, middle, and lower crowns of healthy and well-spaced dominant trees in each plot. Fine roots were sampled using soil cores. Seven soil cores were randomly placed in each plot and each soil core was about 1.0 m away from the nearest tree. A soil corer (9 cm inner diameter) was used to extract soils to a depth of 60 cm (three intervals of 20 cm), which corresponds to the layers of fine root growth in forests (Chang et al., 2012; Zhou and Shangguan, 2007).
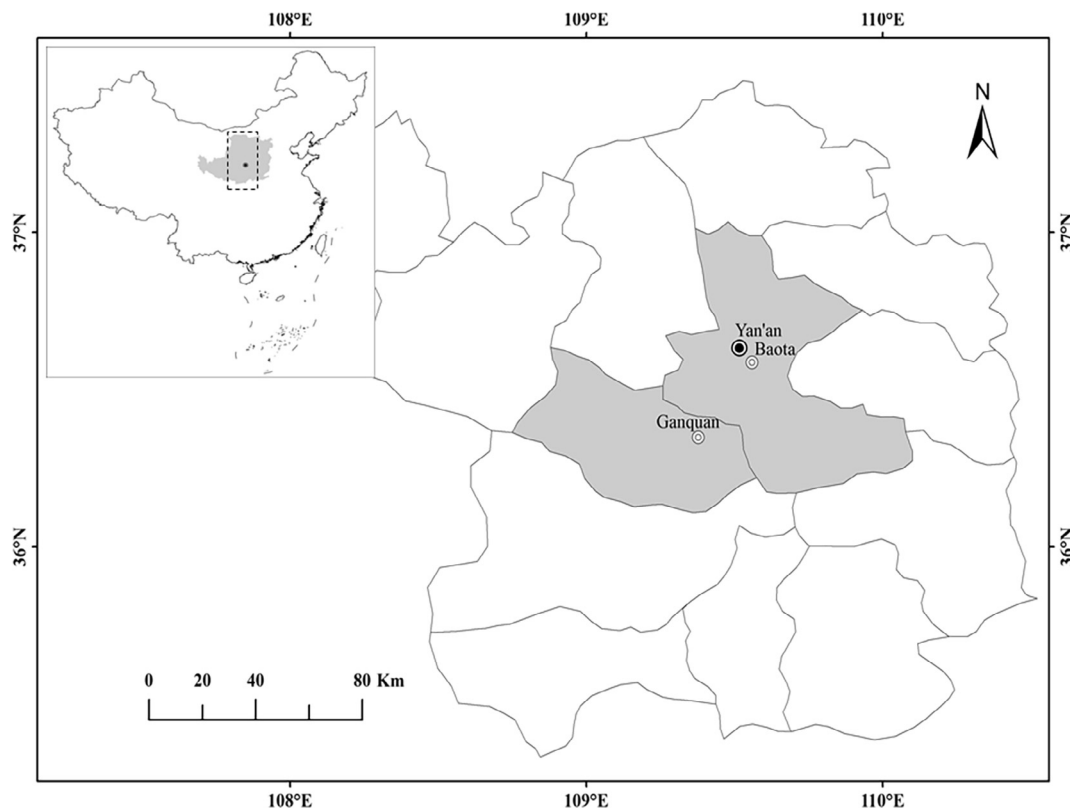

Fig. 1. Locations of sampling stands on the Loess Plateau. The gray area is 5.8×10^3 km^2.

Table 1
Experimental stand characteristics.

Forest type	Stand age (years)	DBH (cm)	Tree height (m)	Elevation (m)	Soil type	Soil pH
Pinus tabulaeformis	10	7.0 ± 0.2[c]	4.4 ± 0.3[c]	1073	Loessial soil	8.54
	25	12.1 ± 0.5[b]	11.4 ± 0.3[b]	1082	Loessial soil	8.18
	40	16.4 ± 1.0[a]	16.4 ± 0.8[a]	1202	Loessial soil	8.13
Robinia pseudoacacia	10	6.4 ± 0.5[b]	6.9 ± 0.4[b]	1276	Loessial soil	8.73
	25	15.3 ± 1.6[a]	9.8 ± 0.9[a]	1352	Loessial soil	8.51
	40	17.3 ± 0.2[a]	10.7 ± 0.3[a]	1342	Loessial soil	8.25

DBH (diameter at breast height) and tree height are means ± standard error (SE), *n* = 3.
Different letters indicate significant differences among stand ages.

Individual soil cores at each plot were treated as subsamples and were averaged by plot. Understory vegetation roots were not separated from the tree roots because the numbers and coverage of understory species were quite low in each stand (see previous section). Root and leaf samples were placed in plastic bags and stored at 4 °C until root separation and/or sample preparation. For root separation, root samples were placed on 0.15 mm mesh sieves and gently washed to collect both live and dead fine roots. Green leaves and fine roots were oven dried at 65 °C to a constant weight and sieved through a 0.15 mm sieve. Retained samples were analyzed for organic C, total N, and P.

Soil samples were collected from three layers (0–20 cm, 20–40 cm, and 40–60 cm) in each soil profile near the root sampling stands (approximately 1.0 kg). Soil samples were air dried and sieved through 1.0 mm and 0.15 mm sieves before determining organic C, total N, and P as well as available N and P. Organic C contents of plant and soil samples were determined using the oil bath-$K_2Cr_2O_7$ titration method (Kalembasa and Jenkinson, 1973). Total N was analyzed with the semi-micro Kjeldahl method (Parkinson and Allen, 1975). For total P analyses, plant and soil samples were digested with H_2SO_4-H_2O_2 and H_2SO_4-$HClO_4$, respectively, then P was determined with colorimetry assays (Parkinson and Allen, 1975). Available soil N was analyzed with the alkali diffusion method (Conway, 1978). To determine available P, soils were extracted with $NaHCO_3$, and P was measured with colorimetry assays (Bao, 2000).

2.3. Data analyses

We used two-way analysis of variance (two-way ANOVA) to analyze effects of stand age, tree species and their interactions on plant stoichiometric characteristics. The effects of stand age on DBH, tree height, soil nutrients, and plant C:N:P stoichiometry was studied using one-way ANOVA. If a difference was significant, multiple post hoc comparisons were made using a Duncan's test. Differences in fine root nutrients among soil layers were tested using a one-way ANOVA and Duncan's test. Analysis of covariance (ANCOVA) was used to test the differences in plant C:N:P stoichiometry between fine roots and green leaves, and age was included as a covariate in an ANCOVA. To examine relationships between fine roots and soil nutrients, we used partial correlation analyses. Factors affecting fine root nutrients were further screened using a stepwise regression method. To investigate the effects of soil C:N:P on plant C:N:P, we performed linear regression analyses. These analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). All statistical significance was set at the 0.05 level.

3. Results

3.1. Stand characteristics and soil nutrients

The DBH and tree height increased significantly with stand age in two plantation forests (Table 1). Soil organic C and total N of both tree species also increased significantly with stand age (Table 2). Stand age was positively associated with total soil P in *P. tabulaeformis* but not *R. pseudoacacia* (Table 2). Available soil N and P of *P. tabulaeformis* and

Available soil P of *R. pseudoacacia* were greater in 10-year stands than 25- and 40-year stands (Table 2).

3.2. Vertical distribution of fine root nutrient concentrations

Fine root C in 10- and 25-year *P. tabulaeformis* stands was greater in surface (0–20 cm) and subsurface (20–40 cm) soil layers than in the bottom (40–60 cm) soil layer (Table 3). Fine root C in 40-year *P. tabulaeformis* stands was greater in the surface soil layer than the bottom layer (Table 3). Fine root N in 10- and 40-year *P. tabulaeformis* was greater in the surface soil layer than the subsurface and bottom layers (Table 3). Fine root N in 40-year *R. pseudoacacia* stands was greater in surface and subsurface soil layers than the bottom layer (Table 3). Fine root P was greater in the surface soil layer than the other soil layers in 25- and 40-year *P. tabulaeformis* and 10-year *R. pseudoacacia* stands (Table 3).

3.3. Leaf and fine root C:N:P stoichiometry

Leaf C and N did not differ significantly with stand age in *P. tabulaeformis* (Fig. 2a and b). In *R. pseudoacacia*, leaf C of 25- and 40-year stands was greater than that of 10-year stands (Fig. 2a). Further, leaf N of 40-year stands was greater than that of 10-year stands (Fig. 2b), whereas leaf P did not change significantly with stand age (Fig. 2c). There were no significant differences among the three stand age classes for leaf C:N, C:P, or N:P (Fig. 3a–c).

Fine root C and N concentrations had similar age-related patterns as those of green leaves (Fig. 2d and e). Fine root P of both tree species differed with stand age (Fig. 2f), whereas fine root C:N did not (Fig. 3d). In *P. tabulaeformis*, fine root C:P of 25-year stands was 20 and 12% higher than those of 10- and 40-year stands, respectively (Fig. 3e). Fine root N:P did not change with stand age (Fig. 3f). In *R. pseudoacacia*, both fine root C:P and N:P of 40-year stands were greater than those of 10- and 25-year stands (Fig. 3e and f).

Leaf and fine root variables were affected by tree species (Table 4). Leaf and fine root N of *P. tabulaeformis* were lower than those of *R. pseudoacacia* in each stand age (Fig. 2b and e), while leaf and fine root C:N between two tree species were in contrast to leaf and fine root N (Fig. 3a and d). Fine roots of *P. tabulaeformis* had greater C:P but lower N:P than fine roots of *R. pseudoacacia* (Fig. 3e and f). The C:N, C:P, and N:P in fine roots and green leaves remained relatively constant with respect to stand age in *P. tabulaeformis* (Table 5; Fig. 3).

3.4. Relationships between plant and soil stoichiometry

In *P. tabulaeformis*, fine root P was negatively correlated with total soil N and positively correlated with total soil P and Available soil N (Table 6). In *R. pseudoacacia*, fine root C was positively correlated with total and Available soil N (Table 6). Based on stepwise regression analysis, fine root P of *P. tabulaeformis* was primarily predicted by total soil P and Available soil N (R^2 = 0.91, *P* = 0.001). Fine root C of *R. pseudoacacia* was primarily predicted by total and Available soil N (R^2 = 0.96, *P* < 0.001). Fine root C:P of both tree species increased

Table 2

Soil organic carbon, total nitrogen, total phosphorus, available nitrogen, and available phosphorus in the 0–60 cm soil layer of two plantation forests.

Forest type	Stand age (years)	Organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})	Available nitrogen (mg kg^{-1})	Available phosphorus (mg kg^{-1})
Pinus tabulaeformis	10	3.4 ± 0.4[c]	0.35 ± 0.06[b]	0.67 ± 0.03[c]	21.9 ± 2.1[a]	2.4 ± 0.2[a]
	25	5.4 ± 0.2[b]	0.68 ± 0.05[a]	0.76 ± 0.01[b]	9.0 ± 1.6[b]	1.4 ± 0.0[b]
	40	6.7 ± 0.0[a]	0.69 ± 0.01[a]	1.01 ± 0.01[a]	8.6 ± 0.4[b]	1.1 ± 0.1[b]
Robinia pseudoacacia	10	1.9 ± 0.0[c]	0.19 ± 0.00[c]	0.94 ± 0.03[a]	18.5 ± 0.9[a]	2.2 ± 0.0[a]
	25	5.0 ± 0.0[b]	0.52 ± 0.02[b]	0.90 ± 0.00[ab]	19.3 ± 0.3[a]	1.5 ± 0.0[b]
	40	6.0 ± 0.3[a]	0.61 ± 0.03[a]	0.86 ± 0.00[b]	20.2 ± 1.2[a]	1.4 ± 0.1[b]

Data are the means ± standard error (SE), *n* = 3.

Different letters indicate significant differences among stand ages.

Table 3

Vertical distribution of fine root nutrient concentrations in two plantation forests.

Forest type	Stand age (years)	Soil layer (cm)	Fine root carbon (g kg^{-1})	Fine root nitrogen (g kg^{-1})	Fine root phosphorus (g kg^{-1})
Pinus tabulaeformis	10	0–20	533.3 ± 17.6[a]	4.72 ± 0.34[a]	0.66 ± 0.03
		20–40	493.3 ± 10.7[a]	3.22 ± 0.28[b]	0.63 ± 0.03
		40–60	419.6 ± 8.5[b]	2.69 ± 0.38[b]	0.67 ± 0.02
	25	0–20	522.8 ± 16.1[a]	5.05 ± 0.60	0.59 ± 0.00[a]
		20–40	503.4 ± 10.7[a]	3.32 ± 0.60	0.49 ± 0.01[b]
		40–60	411.6 ± 5.2[b]	3.45 ± 0.09	0.54 ± 0.03[ab]
	40	0–20	516.8 ± 14.0[a]	5.57 ± 0.37[a]	0.71 ± 0.01[a]
		20–40	455.6 ± 22.0[ab]	4.00 ± 0.30[b]	0.59 ± 0.01[b]
		40–60	414.7 ± 16.9[b]	3.61 ± 0.37[b]	0.45 ± 0.04[c]
Robinia pseudoacacia	10	0–20	404.4 ± 16.5	25.51 ± 2.13	0.99 ± 0.03[a]
		20–40	414.7 ± 16.1	26.02 ± 0.41	0.62 ± 0.08[b]
		40–60	353.8 ± 26.0	22.53 ± 0.84	0.60 ± 0.06[b]
	25	0–20	489.3 ± 15.6	28.85 ± 1.83	0.84 ± 0.04
		20–40	459.7 ± 16.5	29.00 ± 1.91	0.78 ± 0.03
		40–60	440.3 ± 20.5	28.24 ± 1.53	0.75 ± 0.05
	40	0–20	504.5 ± 42.9	33.63 ± 2.43[a]	0.76 ± 0.03
		20–40	464.6 ± 39.2	31.45 ± 0.60[a]	0.68 ± 0.04
		40–60	460.7 ± 9.6	25.71 ± 0.52[b]	0.61 ± 0.07

Data are the means ± standard error (SE), *n* = 3.

Different letters indicate significant differences among soil layers.

linearly with soil C:P (Fig. 4d). In *R. pseudoacacia*, the N:P in fine roots and green leaves increased linearly with total and Available soil N:P (Fig. 4e–h).

4. Discussion

This study showed that fine root C, N, and N:P of *R. pseudoacacia* and fine root P and C:P of both tree species differed with stand age, supporting our hypothesis that the fine root C:N:P stoichiometry of *P. tabulaeformis* and *R. pseudoacacia* forests changes based on stand age. The C, N, and C:N of fine roots showed similar age-related patterns to those of green leaves for both tree species, whereas the P and C:P of fine roots differed from those of green leaves. These results partially support our hypothesis that similar age-related patterns would be observed for leaf and fine root C:N:P stoichiometry in the two species.

4.1. Effect of stand age on leaf and fine root C:N:P

In *R. pseudoacacia* forests, fine root C and N were lowest in 10-year stands, and these values increased significantly in 25- and 40-year stands (Fig. 2d), suggesting that fine root C and N of deciduous species increased with age. In *P. tabulaeformis* forests, fine root C and N did not differ with stand age (Fig. 2d and e), suggesting that age did not affect these nutrient contents in this evergreen species. Species from resource-deficient environments are generally more conservative when responding to changing environmental conditions (Givnish, 2002). Therefore, evergreen species may be less sensitive to nutrient-poor soils than deciduous species (Way and Oren, 2010). Our study suggests that evergreen species utilize a more conservative nutrient-use strategy than

deciduous species when comparing young and mature trees. Fine root P changed significantly with stand age, and this age-related pattern differed between *P. tabulaeformis* and *R. pseudoacacia* (Fig. 2f). These results suggest that fine root nutrients (C, N, and P) in the two species respond differently depending on stand age. For both species, we found that C and N of fine roots had similar age-related patterns to those of green leaves, suggesting that leaf and fine root nutrient characteristics (C and N concentrations) were similar across stand ages. Because green leaves and fine roots are the most active plant organs, it is not surprising that leaf and fine root C and N had similar patterns in this study, as they would be expected to have similar resource-uptake patterns. The trend of increasing leaf and fine root N over time in *R. pseudoacacia* is similar to that of leaf N along chronosequences of *Populus tremuloides* (Yuan and Chen, 2010a) and *Pseudolarix amabilis* (Fan et al., 2015). To our knowledge, there are no published studies of age-related patterns in fine root nutrients for comparison.

In this study, fine root and green-leaf C:N did not change significantly with stand age in *P. tabulaeformis* and *R. pseudoacacia* forests (Fig. 3a and d). However, there is no consensus in the published literature regarding plant C:N ratios relative to stand age. Plant C:N has been reported to increase dramatically (Hooker and Compton, 2003; Yang and Luo, 2011) or not change at all (Clinton et al., 2002) with increasing age. These discrepancies may be due to differences in experimental methods or age sequences (Clinton et al., 2002; Hooker and Compton, 2003; Yang and Luo, 2011). *R. pseudoacacia* had lower fine root C:P in 10- and 25-year stands than in 40-year stands (Fig. 3e). We conclude that the growth rate of fine roots was faster in younger *R. pseudoacacia* stands than in old stands based on the growth-rate hypothesis that C:P is negatively correlated with the growth rate in plants
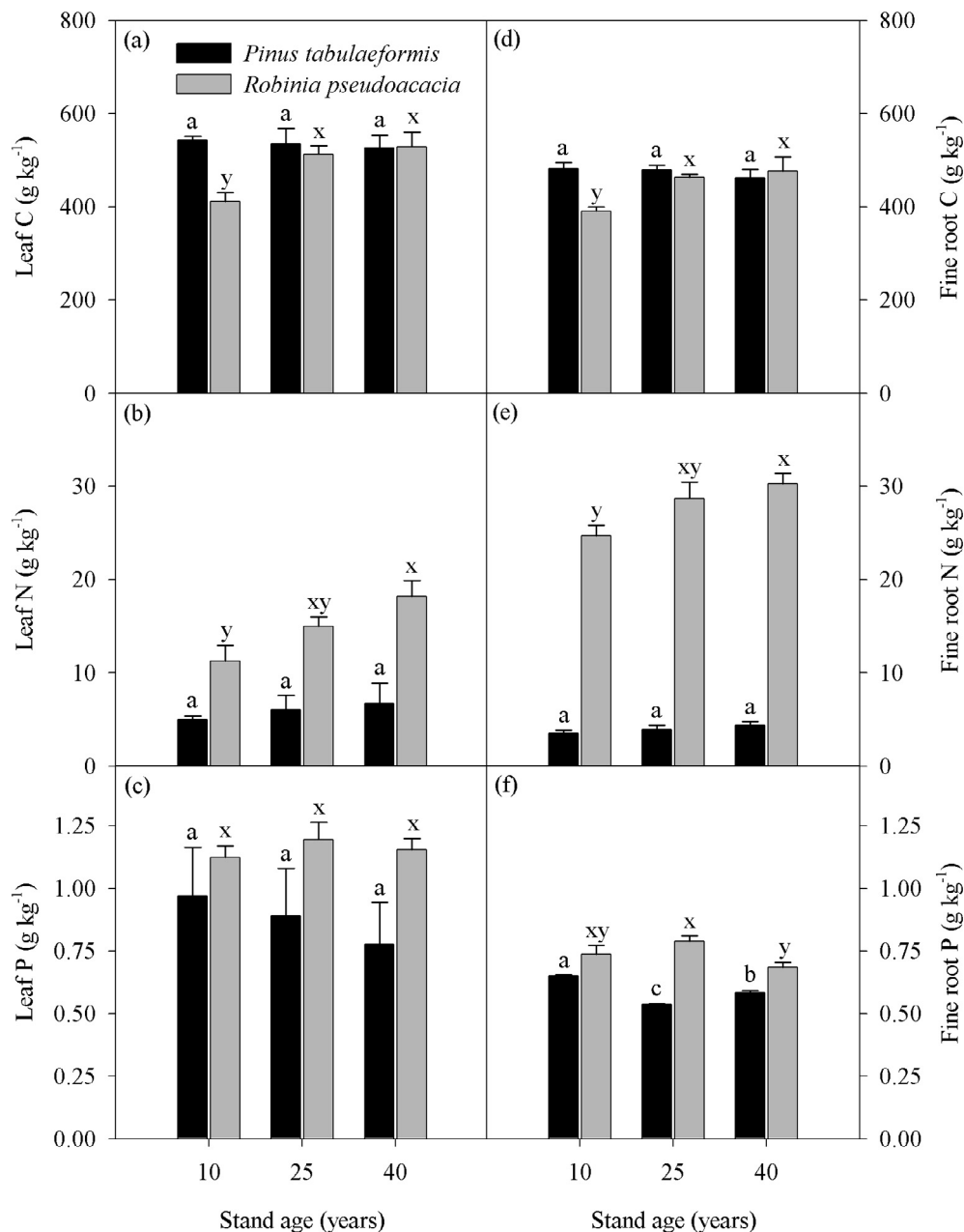
Fig. 2. Stoichiometric characteristics of leaf C (a), leaf N (b), leaf P (c), fine root C (d), fine root N (e), and fine root P (f) in each stand age of two plantation forests. Error bars are the standard error (*n* = 3). Different letters indicate significant differences among stand ages. C, carbon; N, nitrogen; P, phosphorus.

(Aerts, 1996; Agren, 2004; Elser et al., 1996). We found that fine root N:P of *R. pseudoacacia* was greater in 40-year stands than in 10- and 25-year stands, whereas that of *P. tabulaeformis* did not differ significantly with stand age, indicating that the two species differed in their age-related pattern of fine root N:P. Our results also revealed that stand age is a critical factor contributing to fine root C:N:P stoichiometry in *P. tabulaeformis* and *R. pseudoacacia* forests on the Loess Plateau. The underlying mechanisms affecting this stoichiometry are complex. They are likely the result of both physiological (Monaghan et al., 2008) and ecological effects (Bengough et al., 2006; Vitousek et al., 2010; Yuan et al., 2011). The intrinsic changes at the molecular, biochemical, and physiological levels appear to correlate with plant growth, whereas the ecological effects (i.e., soil nutrient availability) are associated with increasing stand age.

The C:N, C:P, and N:P in fine roots and green leaves remained relatively constant across the age sequences in *P. tabulaeformis* forests,

suggesting that this forest ecosystem is relatively stable. Fine roots and leaves are the primary organs for nutrient input and output. Therefore, the elemental stoichiometry in fine roots and green leaves should be consistent to maintain the dynamic nutrient balance (Liu et al., 2010). The C:N was lower and the N:P was higher in fine roots than in green leaves at each stand age of *R. pseudoacacia*, indicating that N is preferentially pooled in fine roots rather than green leaves because fine roots can obtain more N through N-fixing activity (Uselman et al., 2000).

4.2. Variation in fine root C:N:P between tree species

In *P. tabulaeformis* on the Loess Plateau, the ranges of fine root C, N, and P contents were 462.4–482.1, 3.55–4.39, and 0.54–0.65 g kg^{-1}, respectively. In *R. pseudoacacia*, the fine root C, N, and P contents were 391.0–476.6, 24.69–30.26, and 0.68–0.79 g kg^{-1}, respectively. The
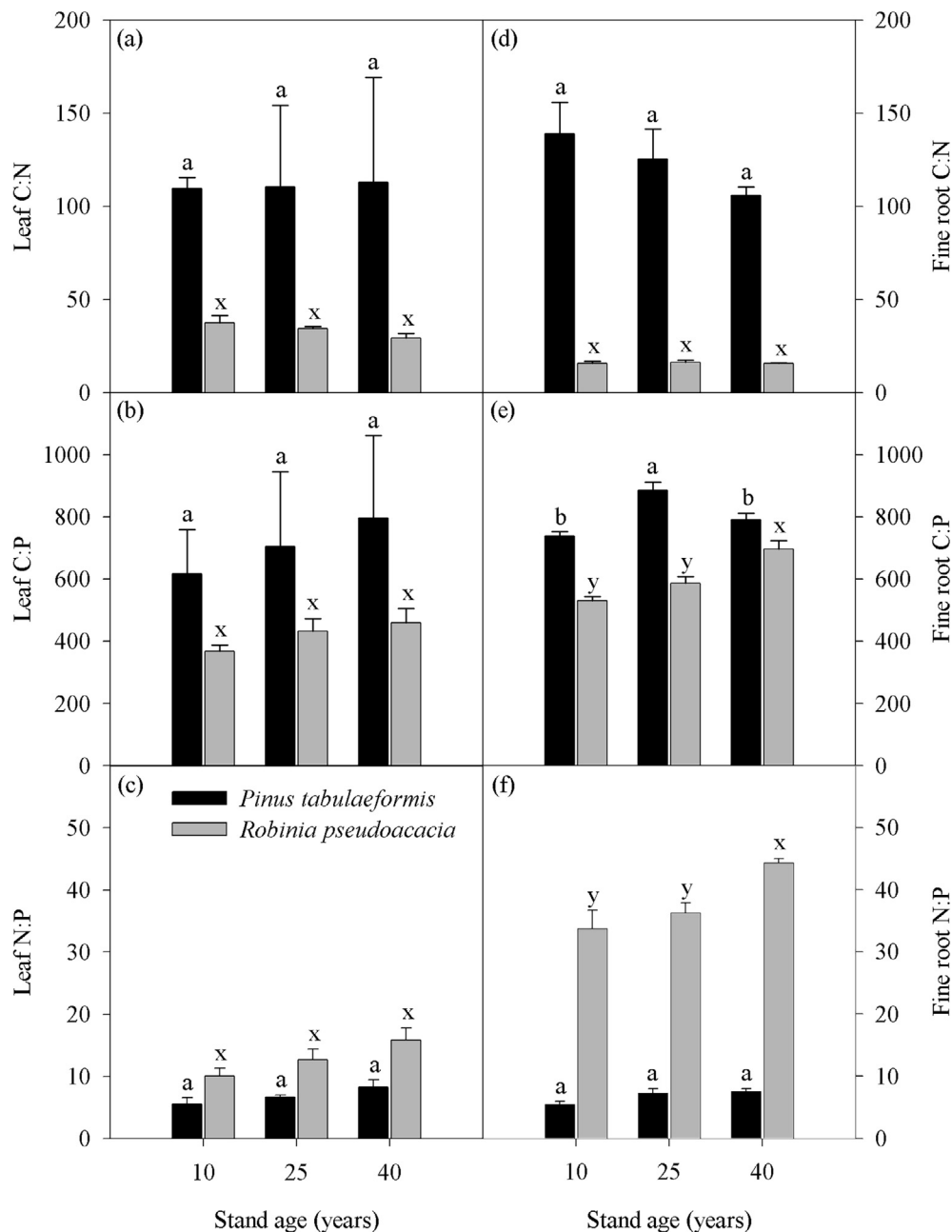
Fig. 3. Stoichiometric characteristics of leaf C:N (a), leaf C:P (b), leaf N:P (c), fine root C:N (d), fine root C:P (e), and fine root N:P (f) in each stand age of two plantation forests. Error bars are the standard error (*n* = 3). Different letters indicate significant differences among stand ages. C, carbon; N, nitrogen; P, phosphorus.

ranges of fine root C contents for *P. tabulaeformis* and *R. pseudoacacia* were similar to the global-scale values reported by Yuan et al. (2011). However, the fine root P contents for both tree species were lower than the global average (1.35 g kg^{-1}). Plant and soil P values are generally coupled in an ecosystem (Hedin, 2004; Specht and Specht, 2010), and the cause for the low fine root P observed in our study might be due to fact that our sites had far lower soil P than the global average (National Soil Survey Office of China, 1997), indicating a P limitation on the Loess Plateau. In comparison, *P. tabulaeformis* had lower fine root N than the global average (20.4 g kg^{-1}), whereas fine root N was higher in *R. pseudoacacia* than the global average (Yuan et al., 2011). This result reflects differences in N-uptake and -use strategies between broad- and needle-leaved species.

Fine root C:N:P differed between the tree species. Fine root C:N and C:P were lower in *R. pseudoacacia* than in *P. tabulaeformis,* likely because broad-leaved species have less N and P deficiency compared with

coniferous species (Yang and Luo, 2011). We found that fine root N and P contents were greater in *R. pseudoacacia* than in *P. tabulaeformis,* supporting the concept that different N- and P-nutrient strategies are utilized in broad-leaved versus coniferous species (Gordon and Jackson, 2000; Yuan and Chen, 2010b), deciduous versus evergreen species (Han et al., 2005), and short-lived, fast-growing versus long-lived, slow-growing species (Aerts, 1996; Foulds, 1993; Güsewell and Koerselman, 2002).

4.3. Effects of soil nutrients on fine root C:N:P

Fine root C, N, and P contents decreased with soil depth and were usually highest in the fine roots of surface soil. Given that topsoil is the primary provider of soil nutrients and water for plant roots to uptake (Chen et al., 2016), this observation is not surprising. The fine root C of *R. pseudoacacia* was significantly and positively correlated with total

Table 4
Statistical analysis of differences in plant carbon (C): nitrogen (N): phosphorus (P) stoichiometry between stand ages and tree species.

Characteristic	Stand age	Tree species	Stand age × Tree species	R^2
Leaf C	**0.018**	**0.026**	**0.041**	0.626
Leaf N	**0.045**	**0.000**	0.279	0.838
Leaf P	0.805	**0.027**	0.704	0.385
Leaf C:N	0.997	**0.007**	0.979	0.465
Leaf C:P	0.704	**0.049**	0.960	0.318
Leaf N:P	0.067	**0.000**	0.527	0.775
Fine root C	**0.048**	**0.039**	**0.021**	0.647
Fine root N	**0.023**	**0.000**	0.089	0.986
Fine root P	**0.035**	**0.000**	**0.002**	0.902
Fine root C:N	0.267	**0.000**	0.278	0.940
Fine root C:P	**0.000**	**0.000**	**0.001**	0.945
Fine root N:P	**0.003**	**0.000**	**0.024**	0.983

Data represent *P*-values unless otherwise indicated. Bold font indicates a significant result.

Table 5
Statistical analysis of differences in carbon (C): nitrogen (N): phosphorus (P) ratios between fine roots and green leaves.

Pinus tabulaeformis	F	P	*Robinia pseudoacacia*	F	P
C	16.738	**0.001**	C	4.877	**0.043**
N	5.855	**0.029**	N	144.266	**0.000**
P	8.802	**0.010**	P	131.098	**0.000**
C:N	0.300	0.592	C:N	102.569	**0.000**
C:P	0.716	0.411	C:P	59.123	**0.000**
N:P	0.012	0.913	N:P	279.608	**0.000**

Bold font indicates a significant result.

Table 6
Partial correlations of fine root carbon (FRC), fine root nitrogen (FRN), and fine root phosphorus (FRP) with Soil organic carbon (SOC), total soil nitrogen (TSN), total soil phosphorus (TSP), Available soil nitrogen (ASN), and Available soil phosphorus (ASP) in *Pinus tabulaeformis* and *Robinia pseudoacacia*.

Characteristic	SOC	TSN	TSP	ASN	ASP
Pinus tabulaeformis					
FRC	0.371	0.115	−0.529	0.021	−0.391
FRN	−0.138	0.018	0.300	0.062	0.455
FRP	−0.307	−0.821[*]	0.739[*]	0.876[**]	0.496
Robinia pseudoacacia					
FRC	0.030	0.763[*]	−0.122	0.821[*]	−0.584
FRN	0.093	0.231	0.430	−0.031	−0.297
FRP	0.469	0.644	−0.502	0.477	−0.652

[*] $P < 0.05$, [**] $P < 0.01$, $n = 9$.

and Available soil N (Table 6). Our stepwise regression analysis confirmed that total and Available soil N impacts the fine root C of *R. pseudoacacia* in the Loess Plateau. Consistent with our findings, Yuan et al. (2011) reported that fine root C was positively associated with soil N. Our results showed increased total soil N and no significant changes in Available soil N with stand age in *R. pseudoacacia*. These different patterns in total and Available soil N are not surprising, as the total amount of N often does not reflect bioavailable N, which primarily comes from mineralization (Yuan and Chen, 2010a). In our study, the stand-age-related increase in fine root N of *R. pseudoacacia* could be the result of an increasing supply of soil N, because fine root N content to some degree reflects soil fertility (Lambers et al., 2008). In the present study, soil N availability did not change with stand age in *R. pseudoacacia*, suggesting that factors other than Available soil N probably affect fine root N. Our analysis showed that fine root P of *P. tabulaeformis* was influenced primarily by total soil P and Available soil N, indicating that soil N availability plays an important role in determining variations in fine root P of *P. tabulaeformis* on the Loess Plateau. This close

relationship between total soil P and fine root P agrees with the result of Yuan et al. (2011). Linear regression analysis showed coupling between soil and plant stoichiometry (i.e., soil C:P and fine root C:P of both tree species; total and Available soil N:P with leaf and fine root N:P, respectively, of *R. pseudoacacia*) (Fig. 4), indicating that fine root and leaf stoichiometry are tightly linked to soil stoichiometry. This is consistent with previous studies of other forest ecosystems (Fan et al., 2015; Liu et al., 2010). Notably, fine root stoichiometry is more directly affected by soil nutrients (Gordon and Jackson, 2000; Yuan et al., 2011), because the effects of nutrient deficiency are first apparent in roots and subsequently in leaves (Liu et al., 2010).

The N:P thresholds proposed by Koerselman and Meuleman (1996) theorized that N:P < 14 indicates N limitation; N:P > 16 indicates P limitation; and between 14 and 16 indicates co-limitation. In this study, the fine root and leaf N:P values of *P. tabulaeformis* suggest that N is limited in 10-, 25-, to 40-year *P. tabulaeformis* stands on the Loess Plateau. The leaf N:P of *R. pseudoacacia* suggest that N is limited in 10- and 25-year stands, whereas N and P are limited in 40-year stands, supporting the hypothesis that P becomes increasingly limiting relative to N over time in forests (Fan et al., 2015; Wardle et al., 2004). The Available soil N did not decline, but the Available soil P significantly declined with stand age in *R. pseudoacacia* (Table 2), which may directly demonstrate that P limitation increased with stand age in *R. pseudoacacia* on the Loess Plateau. Low P availability in soils is due primarily to the fact that soil minerals are gradually depleted and phosphate is transformed in long-term forest stands (Crews et al., 1995). The N:P in fine roots of *R. pseudoacacia* might not be a suitable indicator of nutrient limitation because the N-fixing activity occurring in roots (Uselman et al., 2000) contributes toward N availability.

5. Conclusions

To our knowledge, this is the first study to explore how stand age influences fine root C:N:P stoichiometry in different forest types in a warm temperate zone. Our results showed that fine root C, N, and N:P of *R. pseudoacacia* were greater in old than young stands. However, fine root C, N, and N:P of *P. tabulaeformis* did not vary with stand age, suggesting that evergreen species utilize a more conservative nutrient-use strategy during aging. The age-related patterns of C, N, and C:N in fine roots were similar to those in green leaves. The positive correlations between fine root nutrient stoichiometry and soil nutrients suggest a tight coupling between fine root and soil nutrients. These results could improve our understanding of fine root C:N:P stoichiometry with respect to stand age in forests. Further studies are needed to investigate temporal changes in fine root C:N:P stoichiometry across longer age sequences, particularly for fine roots because of functional differences in roots with diameters of ≤2 mm (Joslin et al., 2006; Pregitzer et al., 2002). Finer roots (< 0.5 mm) primarily function in nutrient uptake, whereas coarser roots (0.5–2 mm) primarily function in nutrient transport.

Conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.
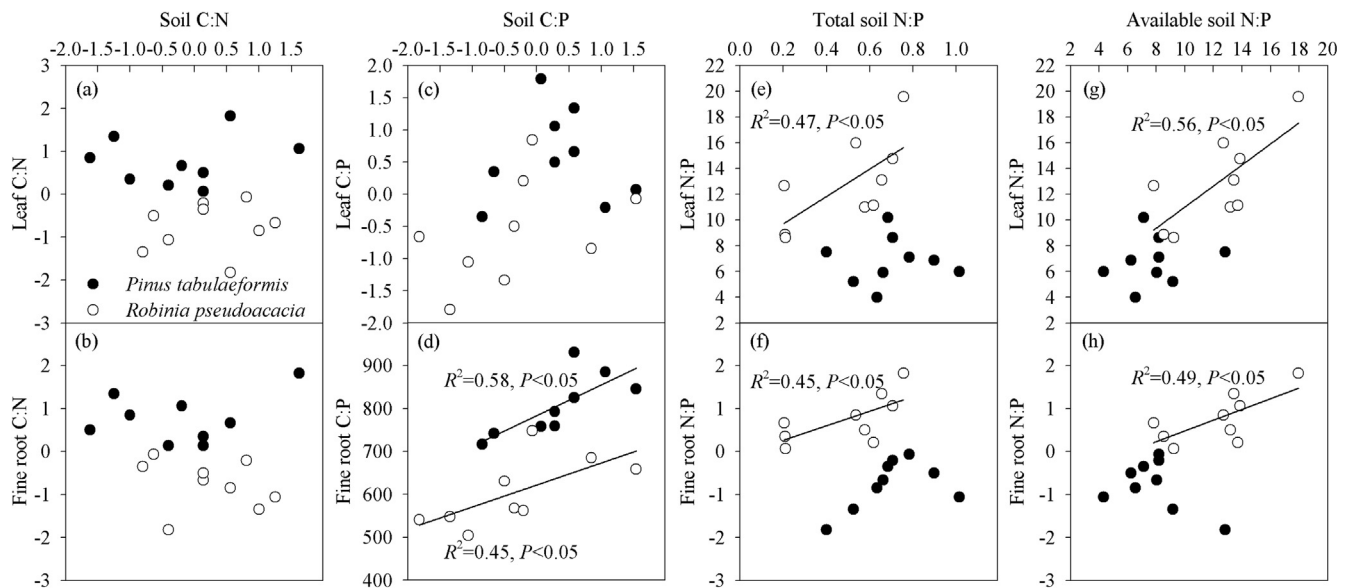
Fig. 4. Soil C:N in relation to leaf C:N (a) and fine root C:N (b). Soil C:P in relation to leaf C:P (c) and fine root C:P (d). total soil N:P in relation to leaf N:P (e) and fine root N:P (f). Available soil N:P in relation to leaf N:P (g) and fine root N:P (h). Solid black lines represent the fitted linear regressions reaching a significant level. Ratio values are transformed when needed to meet the normality assumption. C, carbon; N, nitrogen; P, phosphorus.

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