

Coupling of plant and soil C:N:P stoichiometry in black locust (*Robinia pseudoacacia*) plantations on the Loess Plateau, China

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Abstract

Key message C:N remained constant, but C:P and N:P were higher in the 37-year-old stands. The correlations were linked by P and N:P among plant, but N:P and C:P between soil and plant.

Abstract Carbon (C), nitrogen (N), and phosphorus (P) stoichiometries are critical indicators of interlinked biogeochemical cycles in terrestrial ecosystems. However, the linking manner between plant and soil in plantations remains unclear. We examined the variations in the concentrations of C, N, and P, and their ratios in plant tissues, litter and soils in an age sequence of 9-, 17-, 30- and 37-year-old black locust (*Robinia pseudoacacia*) plantations afforested on the Loess Plateau, China. The C:N ratios in plant tissues and soil remained relatively stable across age sequence. However, the C:P and N:P ratios were significantly higher in the 37-year-old stands than in those of the previous growth stages because of the decreased P concentrations in the plant tissues and the increased C and N concentrations in the soils. The strong positive correlations among plant tissues were tightly

linked by elemental P and the N:P ratios. Moreover, the N:P and C:P ratios between soil and plant tissues were strongly correlated. The age-related increase of N:P ratios indicated that the growth of black locust plantations in the Loess Plateau becomes progressively P limited when aging. Therefore, it is necessary to enhance the forest structure and to reorganize ecological functions of plantations in the Loess Plateau when they appear to decline under nutrient limitation as a result of long-term development.

Keywords Ecological stoichiometry · Plant–soil interaction · N:P ratio · Nutrient limitation

Introduction

Carbon (C), nitrogen (N), and phosphorus (P) are the three major elements that comprise living organisms, and the key characteristics of organisms and ecosystems are determined by the dynamics of these element ratios. Above- and below-ground ecological components are tightly linked and their interactions greatly affect ecosystem processes and properties (Wardle et al. 2004a). Therefore, further insights into the C:N:P stoichiometry relationships between plants and soils are suggested as a major ecological indicator for understanding ecosystem function and processes (Elser and Hamilton 2007; Elser et al. 2000; Zhao et al. 2015). Numerous studies have greatly advanced our understanding of the spatial variability of C:N:P ratios in terrestrial ecosystems (Cleveland and Liptzin 2007; Reich and Oleksyn 2004). Well-constrained C:N ratios were found in forests in leaf, litter, and mineral soil compartments at the global level (Cleveland and Liptzin 2007; Han et al. 2005; Tian 2010; Yang and Luo 2011). N:P ratios in leaves and

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roots decrease with increasing latitude, which reflects the change in soils from high-N and low-P levels to low-N and high-P levels (McGroddy et al. 2004; Richardson et al. 2008; Yuan et al. 2011). However, a study on Chinese terrestrial plant species found no significant change in the leaf N:P ratio with increasing latitudes (Han et al. 2005).

Our understanding of the temporal variability of ecological stoichiometry is much less developed than that of its spatial patterns (Agren 2008). It has been suggested that ecological patterns occurring over time may differ from those observed across space (Fisher et al. 2010; Yang and Luo 2011). More recent studies allow for the quantification of the temporal patterns of C:N:P stoichiometry in natural ecosystems (Parfitt et al. 2005; Perez et al. 2014; Reed et al. 2011; Tibbets and Molles 2005). Most of these studies supported Walker and Syers (1976) model of soil development that plant growth is limited by N at younger sites, co-limited by N and P at intermediate-aged sites, and P limited at older sites (Hayes et al. 2014; Reed et al. 2011). Data from secondary forests around the world demonstrated that the plant C:N ratios increased significantly with increasing age (Yang and Luo 2011). However, Clinton et al. (2002) observed that the plant C:N ratios did not change significantly over a 150-year-old *Nothofagus solandri* chronosequence in New Zealand. Wardle et al. (2004b) found that N:P and C:P ratios of the humus and litter significantly increased toward the latter phase for six long-term chronosequences in Australia, Sweden, Alaska, Hawaii, and New Zealand, indicating increasing P limitation over time. Therefore, climate, topography, nutrient redistribution, vegetation composition, species diversity, and plant physiological constraints have been used to explain the inconsistent C:N:P results between plants and soils in different regions (Agren and Weih 2012; Reich and Oleksyn 2004; Townsend et al. 2007).

Human activities on land use change (i.e., afforestation) can alter the bio-geochemical cycling of C, N, and P, obscuring relationships between soil nutrient status and the original vegetation (Gao et al. 2014; Parfitt et al. 2005). Most studies have focused on plant and soil stoichiometries of natural forests in which the nutrient status possibly differs from that of forest plantations (Agren 2008; Elser et al. 2010; Liao et al. 2012). Chen et al. (2005) reported that soil C and N concentrations in plantations were lower than those found in natural forests. Gao et al. (2014) found that C:N ratios for surface soils generally increased over time but declined in deeper soil layers while both C:P and N:P ratios declined significantly over time in different land use types in southern China. A global synthesis of soil C:N stoichiometry following afforestation showed that C:N ratios remained constant in the mineral horizon but significantly decreased in the organic horizon as stand age increased (Xu et al. 2016). However, a study that analyzed

the plantation soil C, N, and P concentrations under the Grain-to-Green Program in China found that soil C:N ratios increased during the first 10 years after afforestation and after 20 years but decreased from 10 to 20 years, while soil C:P ratios decreased over the first 10 years and then increased and N:P ratios constantly decreased (Zhao et al. 2015). However, considerable uncertainty still surrounds the variation in C:N:P ratios following afforestation (Xu et al. 2016). Knowledge of C:N:P stoichiometry following afforestation could advance our understanding of the nutrient elements coupling between above- and below-ground ecological components to illustrate how biogeochemical cycles change during stand development in tree plantations (Yang and Luo 2011).

A large-scale government program on the Chinese Loess Plateau has been implemented in order to create forest plantations, control soil erosion, and restore degraded ecosystems. Approximately 5.2 million ha of plantations were established on the Loess Plateau from 1999 to 2008. The tree species often chosen was black locust (*Robinia pseudoacacia*) because of its fast growth rate and ability to fix nitrogen, thereby allowing it to thrive in relatively infertile soils. Previous studies have surveyed the biomass and C storage, soil organic C, and soil chemical and microbiological properties of black locust plantations to understand the ecological restoration process after afforestation on the Loess Plateau (Li et al. 2013, 2015; Lu et al. 2013; Qiu et al. 2010; Tateno et al. 2007; Wang et al. 2012). However, few studies have examined the correlations between C:N:P elements among major components of black locust plantations over an age sequence following afforestation. Results from such studies may provide new insights for assessing the ecological success of restoration by afforestation on the Loess Plateau. In this study, we examined the temporal patterns and interactions of the C:N:P ratios in black locust tree tissues, understory vegetation, and litter and soil in an age sequence of 9-, 17-, 30- and 37-year-old black locust plantations afforested on the Loess Plateau to test following three hypotheses: (1) the nutrient concentrations and stoichiometries in black locust trees tissues, understory vegetation, and litter display tissue- and age-specific patterns, (2) different change patterns of soil nutrient concentrations leading to depth- and age-specific soil nutrient stoichiometries, and (3) different nutrient concentrations and stoichiometries correlations exist among plant tissues and between plant tissues and soil. The results of this investigation may provide a crucial complement to explore the relationship between ecosystem stoichiometry and plant function or environmental adaptation mechanism, especially about the ecological restoration process after afforestation on the Loess Plateau, China.

Materials and methods

Study site and sampling

This study was conducted in the Zhifanggou Watershed, located in the Yan River basin of Ansai County (36°46′28″–36°46′42″N, 109°13′03″–109°16′46″E, 1010–1431 m a.s.l), North Shaanxi Province, China. The study area is characterized by a temperate, semi-arid climate. The mean annual temperature in the watershed ranges from 7.7 to 10.6 °C and the mean frost-free period is 157 days. The mean annual precipitation is 505 mm, about 70% of which falls between July and September. The soils are classified as Calcic Cambisols according to the soil map of the world (FAO 1974). The most common soil in this watershed develops on loess soil and has a texture with sand, silt, and clay contents of 65, 24 and 11%, respectively.

In the whole watershed area, black locust plantations aged 9, 17, 30, and 37 years old were randomly selected with the guidance of local forestry staff and village elders. Each age class was represented by three plantations and one plot (20 m × 20 m) was established in each plantation. Therefore, the total twelve plots were randomly selected in the whole watershed with few differences among the sites in regard to aspect, gradient, elevation, and previous farming practices (Table S1). All sampling was carried out in August 2011. Diameter at breast height (DBH) and tree height were measured for all of the trees in each plot. C, N, and P concentrations were estimated in various tree components (i.e., leaves, branches, stems, and roots) by sampling from five trees representing the stand-specific DBH range in each plot. Living branches with leaves were collected using a pole pruner from the upper, middle, and lower parts of the canopy in all four cardinal compass directions, and divided into pooled samples of branches and leaves. Stem samples were obtained at breast height of the sampled trees by using an increment borer. Roots (<2 mm in diameter) were collected from the upper 20-cm soil layer in undisturbed soil cores (9.8 cm in diameter) at a distance of 50 cm from the sampled trees in all four cardinal directions. In the understory layer (where only the herb layer was present), litter biomass was randomly collected from five 1 m × 1 m subplots at each plot. The above- and below-ground parts of all herbs were harvested through destructive sampling from these subplots. The various tissue samples from the subplots were combined to give plot samples. Subsequently, all of the weighed samples of the tree components, herb parts, and litter were oven-dried to a constant mass at 65 °C and reweighed. The dried samples were ground and used to determine plant C and N concentrations using an elemental analyzer (Carlo Erba 1106, Milan, Italy) based on

modification of the classical Pregl and Dumas method (combustion temperature of 1030 °C, atmosphere of oxygen) using 0.5–1.0 mg of sample per analysis. Total P was determined by the HClO₄–H₂SO₄ colorimetric method (Parkinson and Allen 1975).

In each plot, six undisturbed soil columns were removed from the upper 60-cm soil layer by augering (5-cm diameter) at three depth intervals: 0–20, 20–40, and 40–60 cm. All of the soil samples were sieved through a 2-mm mesh, and roots and other coarse debris were removed. The sieved soil samples were then air-dried for the determination of the soil chemical properties. C concentration was determined by the Walkley–Black K₂Cr₂O₇–H₂SO₄ method (Nelson et al. 1996); total N concentration by the Kjeldahl method (Bremner and Mulvaney 1982); and total P by the HClO₄–H₂SO₄ colorimetric method (Parkinson and Allen 1975). The C:N, C:P, N:P, and C:N:P ratios of the different ecological components were expressed as molar ratios, and not as mass ratios.

Data analysis

All of the statistical analyses were performed with SPSS software (version 20.0, SPSS Inc, Chicago, IL, USA). All of the data were logarithmically transformed to meet the assumptions of normality and homogeneity when needed. Two-way ANOVA were conducted in order to identify statistically significant differences among the C, N, and P concentrations, and their ratios, for the major ecosystem components and ages. Least Squares Difference (LSD) post hoc tests were conducted to identify significant differences between means. Relationships of C, N, and P concentrations and of their ratios between the major ecosystem components were determined by Pearson correlation analyses. The level of statistical significance was set at $P < 0.05$.

Results

Patterns of tree C, N and P concentrations and C:N:P ratios

The C concentrations were similar among stand age and significantly different C concentrations among tree tissues were observed only in the 17-year-old stand ($P < 0.05$) (Table 1, Fig. 1a). In contrast, the N concentration in the branches was significantly different among the stand ages and significant differences among tree tissues were commonly found in three of the four stand age groups (i.e., 9-, 17-, and 37-year-old stands; Table 1; Fig. 1b). The P concentration differences among tree tissues were found in

Table 1 *F* and *P* values for the effects of plant tissues, plantation age, and soil depth on plant and soil C:N:P stoichiometric characteristics in black locust plantations on the Loess Plateau, China

Component	Factor	<i>F(P)</i> value					
		C	N	P	C:N	C:P	N:P
Tree	Tree tissues	6.05 (0.002)	20.9 (<0.001)	24.7 (<0.001)	43.6 (<0.001)	98.1 (<0.001)	184 (<0.001)
	Plantation age	1.88 (0.15)	0.16 (0.92)	93.8 (<0.001)	2.2 (0.12)	404 (<0.001)	398 (<0.001)
	Tree tissues × plantation age	1.46 (0.21)	1.80 (0.11)	6.47 (<0.001)	4.36 (0.001)	92 (<0.001)	103 (<0.001)
Herb	Herb tissues	6.40 (0.02)	0.03 (0.87)	25.5 (<0.001)	0.22 (0.64)	17.9 (0.001)	2.36 (0.15)
	Plantation age	4.19 (0.02)	2.56 (0.09)	25.9 (<0.001)	2.19 (0.13)	67.7 (<0.001)	7.70 (0.002)
	Herb tissues × plantation age	7.07 (0.03)	2.54 (0.09)	0.46 (<0.72)	1.10 (0.38)	0.36 (0.79)	2.18 (0.13)
Litter	Plantation age	17.6 (0.001)	9.7 (0.005)	99.0 (<0.001)	4.72 (0.03)	0.67 (0.59)	3.61 (0.07)
Soil	Soil depth	7.33 (<0.001)	19.0 (<0.001)	0.96 (0.39)	0.32 (0.73)	17.7 (<0.001)	19.1 (<0.001)
	Plantation age	7.84 (0.001)	21.3 (<0.001)	2.70 (0.07)	2.29 (0.10)	9.62 (<0.001)	25.3 (<0.001)
	Soil depth × plantation age	4.09 (0.006)	2.64 (0.41)	1.22 (0.33)	1.10 (0.39)	4.26 (0.005)	2.22 (0.07)

the three older age groups (i.e., 17, 30, and 37 years old), and the leaves had the highest P concentration. The P concentrations of 37-year-old trees were significantly lower than those of the other ages ($P < 0.001$, Table 1; Fig. 1c).

The C:N ratio in the branches was significantly higher than in other tree tissues across all age groups, and the C:N ratio difference in the branches was significant only between 9 years and the rest stand ages (Fig. 1d). Both age and tissue type had significant effects on the C:P and N:P ratios ($P < 0.001$, Table 1). The highest C:P and N:P ratios were both observed in the roots of the 37-year-old trees (Fig. 1e, f). On average, the tree tissue C:N:P ratio for the black locust plantations on the Loess Plateau was 611:17:1.

Patterns of C, N, and P concentrations and ratios in herb and litter layers

Significantly different C concentrations were only observed in the 37-year-old stand for above- and below-ground herb and litter layers (Table 1; Fig. 2a). The below-ground parts of the herb tended to have higher N concentrations than the above-ground herb and litter layers, and these were significantly different only for the 17-year-old stand ($P < 0.001$, Fig. 2b). The N concentrations of the below-ground herb and litter layers greatly decreased in the 37-year-old stand. The P concentration was highest in the above-ground herb tissues of the 9- to 30-year-old stands, but the concentrations in above- and below-ground herbs and litter significantly decreased in the 37-year-old stands (Fig. 2c). The C:P and N:P ratios of the above- and below-ground herb in the 37-year-old stand were significantly higher than those in the younger age groups (Fig. 2e, f). The C:P and N:P ratios in litter were constant among the age groups (Table 1; Fig. 2e, f). The mean C:N:P ratios for above- and below-ground herb and litter layers were 435:17:1, 595:25:1, and 486:16:1, respectively.

Patterns of C, N, and P concentrations and ratios in soil

The C and N concentrations in the upper soil layer (0–20 cm) were significantly higher than those in the two deeper soil layers, and the values significantly increased as the stands increased in age from 30 to 37 years old (Table 1; Fig. 3a, b). However, the P concentration was similar within the 60-cm soil profile and remained relatively stable across age sequence (Table 1; Fig. 3c). The C:P and N:P ratios in the upper soil layer (0–20 cm) were significantly higher than in the two deeper soil layers in the 37-year-old stand (Table 1; Fig. 3). From 30 to 37 years old, significant increases in the ratios were observed for C:P in the upper soil layer and N:P in all three soil layers. On average, the C:N:P ratio in the 60-cm soil profile under black locust plantations was 15:2:1, while in the upper soil layer (0–20 cm) it was 22:2:1.

Significant positive correlations between the plant components were observed for the P concentrations and the ratios of C:P and N:P (Table 2). The significant positive correlations between the litter and other vegetation components were linked by their P concentrations and N:P ratios (Table 2). In contrast, between soil and other components, there were significant positive correlations for the C:P and N:P ratios (Table 2).

Discussion

In this study, the dynamics of C, N, and P concentrations and their ratios displayed tissue- and age-specific patterns in black locust plantations on the Loess Plateau, China (Figs. 1, 2, 3). Previous studies report that the increasing ratio of C:N in wood was higher than in leaves or roots with stand age, and explained that the increased C:N ratios

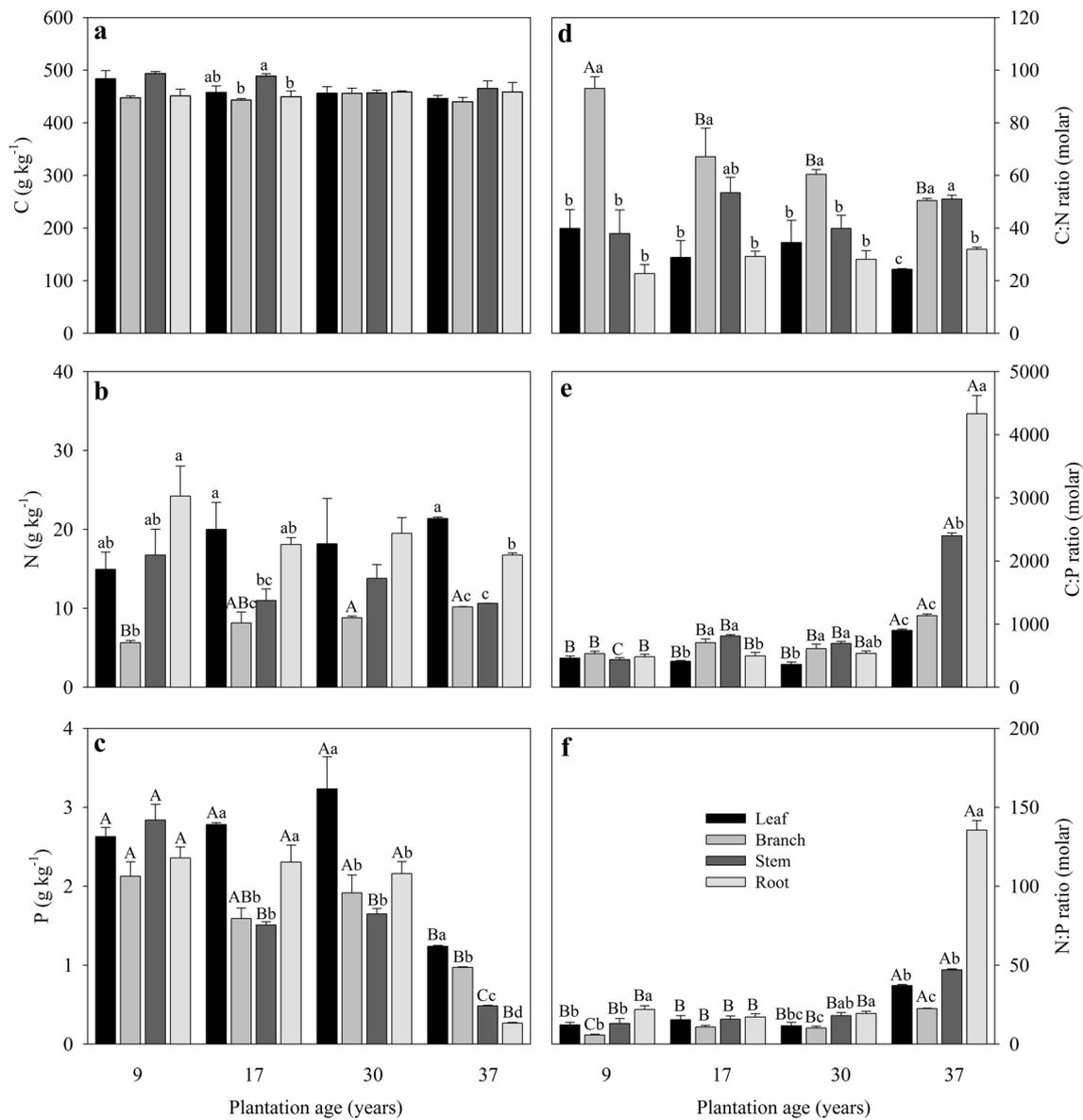


Fig. 1 Stoichiometric characteristics of tree tissues C, N, and P for black locust plantations. Bars indicate standard errors ($n = 3$). Different lowercase letters on the bars indicate significant differences

among tissues for the same age group, while the different uppercase letters indicate significant differences among the age groups for the same tissue type

attributed to changes in the wood structural tissues of the tree during stand development rather than by limitations due to photosynthesis (Hooker and Compton 2003; Yang and Luo 2011). By contrast, the tree C:N ratios remain constant with stand age except for the decreased C:N in the branches in this study (Fig. 1d). Stable C:N ratios in the rest tissues apart from branches may be an indicator of trees suffering from a certain degree of deficiency due to a lack of an abundant supply of photosynthetic products for tree structural construction. The C:N ratios in the herbs and in the soil also remained remarkably stable when considered according to age sequence, which is consistent with results reported for secondary forests worldwide (Yang and

Luo 2011). However, Hooker and Compton (2003) observed that the increased soil C:N ratios in the 0–20 cm soil were controlled by decreasing soil N and in the subsoil were primarily driven by increasing soil C during the first 115 years of forest development in Rhode Island, USA.

In contrast to C and N, P concentrations in plant tissues significantly decreased at the older stage in the 37-year-old stand while remaining relatively stable in the soil during the stand development. As a consequence, C:P and N:P ratios of the various ecosystem components significantly increased at the 37-year-old stand. An increasing number of studies have used the leaf N:P ratio to indicate soil nutrient limitation at the plant community level (Craine

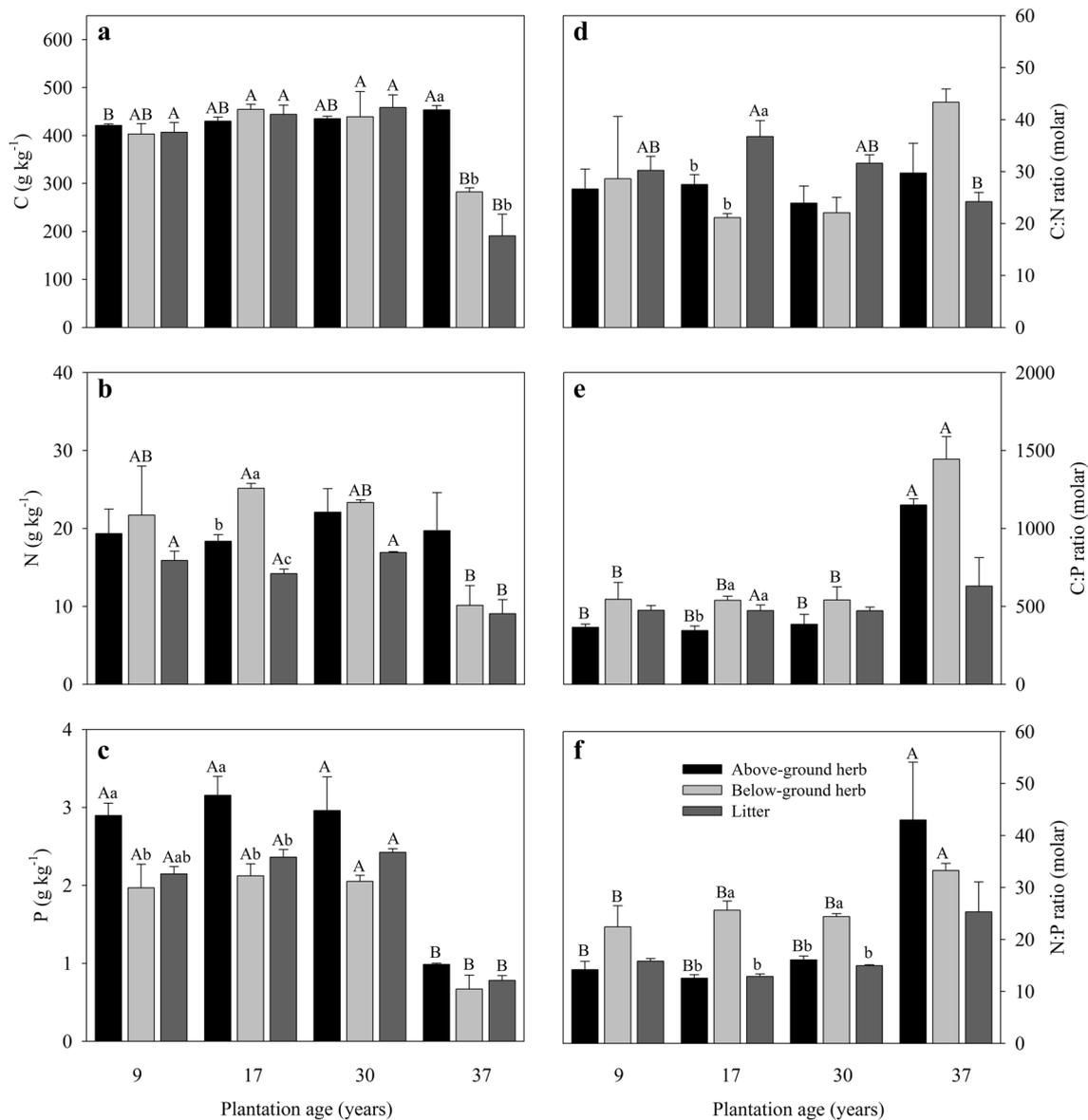


Fig. 2 Stoichiometric characteristics of C, N, and P in the above- and below-ground herb and litter layers of black locust plantations. Bars indicate standard errors ($n = 3$). Different lowercase letters on the

bars indicate significant differences among tissues for the same age group, while the different uppercase letters indicate significant differences among the age groups for the same tissue type

et al. 2008; Gusewell 2004; Mamolos et al. 2005; Reich and Oleksyn 2004; Richardson et al. 2008). Based on these estimates, the increased leaf N:P ratio with increasing age reflects a shift from N to P limitation, a mechanism commonly observed in short- and long-term chronosequences (Fan et al. 2015; Gusewell 2004; Hayes et al. 2014). Hence, the leaf N:P ratio implies that the soil under the 37-year-old stand was P limited relative to N. However, a study in southern Chile found that leaf C:N and C:P ratios increased but N:P ratios remained constant throughout the development of a forest on a 400-year soil chronosequence (Perez et al. 2014). Although previous nutrient-addition glasshouse studies support a shift from N to P limitation by

using leaf N:P as an indicator, quantifying the soil N or P limitation solely through the use of the leaf N:P ratio presents challenges because of the limited amount of data and partial understanding of the processes involved, especially when leaves of different species are compared (Craine et al. 2008; Goll et al. 2012; Townsend et al. 2007). Future field fertilization experiments could help to test the hypothesis of leaf N:P as an indicator for quantifying the soil N or P limitation (Hayes et al. 2014). Moreover, Li et al. (2012) suggested that a more complete understanding of nutrient limitations would include measuring not only the N:P ratios in plants but also those in the soil and in the soil microbial biomass.

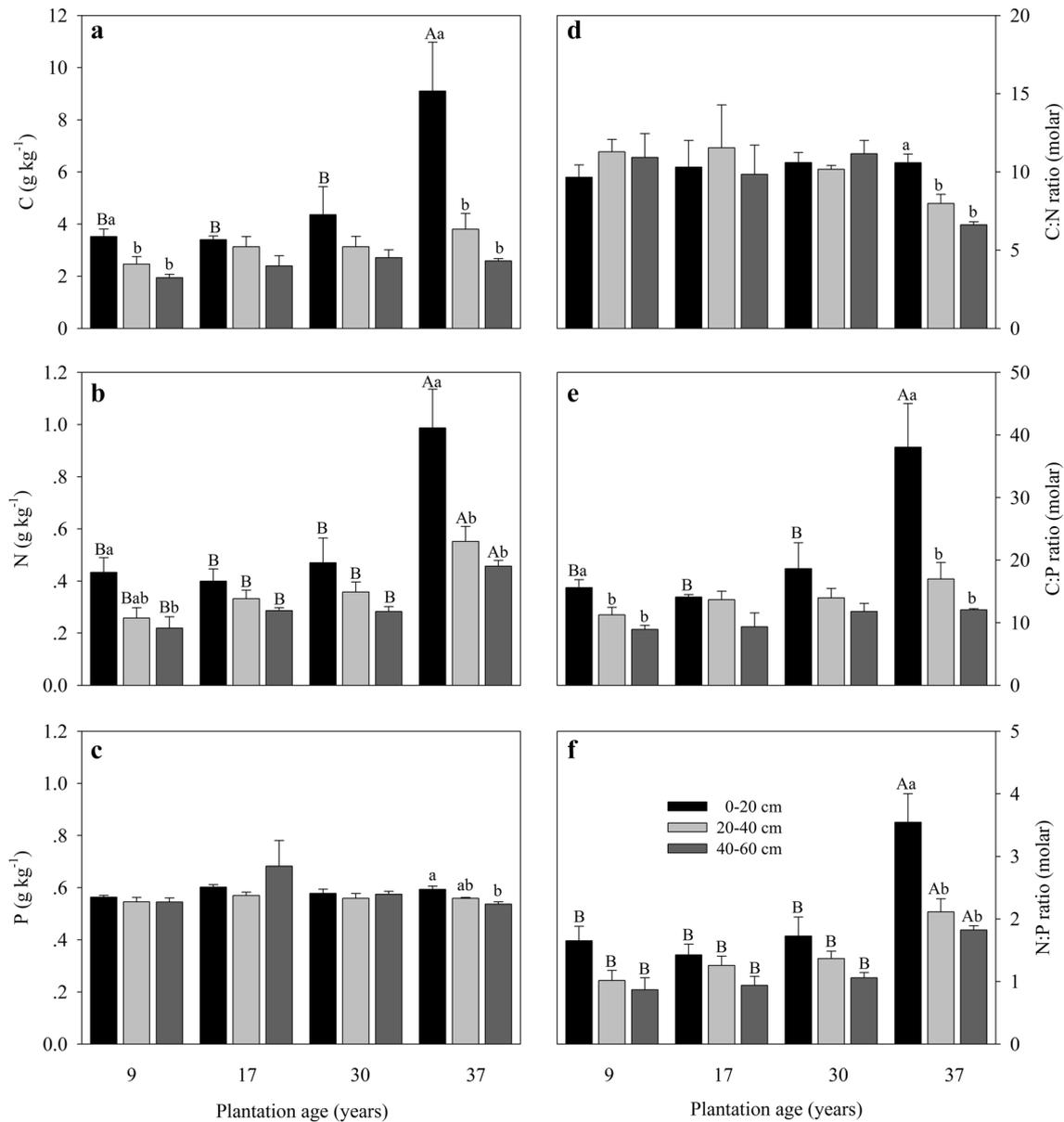


Fig. 3 Stoichiometric characteristics of soil C, N, and P within three layers of a 60-cm soil profile under black locust plantations. Bars indicate standard errors ($n = 3$). Different lowercase letters on the

bars indicate significant differences among tissues for the same age group, while the different uppercase letters indicate significant differences among the age groups for the same tissue type

The lowest P concentration and the highest C:P and N:P ratios, which were observed in the tree roots in the 37-year-old stand, indicated that more P translocation from the roots to other tissues, like leaves, was taking place. This result was contrary to the growth rate hypothesis whereby faster growing tissues such as roots should need relatively more P to support protein synthesis and thus would exhibit lower tissue N:P and C:P ratios (Yuan et al. 2011). Elser et al. (2010) hypothesized that leaf P concentration should decrease with increasing plant size and, thus, large plants would have higher leaf N:P ratios. Similar observations have been made during plant growing seasons (Agren

2008; Tibbets and Molles 2005). At the global scale, the leaves C:N:P ratio was similar to that of the fine roots (i.e., 1158:24:1) (Jackson et al. 1997). However, in our study, the C:N:P ratio in the leaves and roots was not similar to those reported for natural forests in previous studies (Gordon and Jackson 2000; Jackson et al. 1997; McGroddy et al. 2004), supporting the notion that the N₂-fixing ability of black locusts caused a high degree of discrepancy between resources and consumption during the growth in our study area, especially at the older stage.

Litter constitutes a major portion of nutrient cycling between plants and soils (Agren et al. 2013; McGroddy

Table 2 The correlation matrix of the concentrations of C, N, and P, and their ratios between the major ecosystem components in black locust plantations on the Loess Plateau, China

	Leaves	Branches	Stems	Roots	Above-ground	Below-ground	Litter
Tree branches	P (0.79) ^a C:P (0.89) N:P (0.91)						
Tree stems	P (0.61) C:P (0.94) N:P (0.92)	P (0.81) C:P (0.95) N:P (0.93)					
Tree roots	P (0.85) C:P (0.96) N:P (0.95)	P (0.79) C:P (0.93) N:P (0.92)	P (0.79) C:P (0.98) N:P (0.96)				
Above-ground herb parts	P (0.72) C:P (0.94) N:P (0.79)	P (0.65) C:P (0.89) N:P (0.78)	P (0.66) C:P (0.96) N:P (0.82)	P (0.90) C:P (0.97) N:P (0.83)			
Below-ground herb parts	P (0.84) C:P (0.95) N:P (0.71)	P (0.77) C:P (0.88) N:P (0.72)	P (0.69) C:P (0.92) N:P (0.67)	P (0.86) C:P (0.91) N:P (0.73)	P (0.82) C:N (0.62) C:P (0.95) N:P (0.59)		
Litter	P (0.91) N:P (0.72)	P (0.78) N:P (0.62)	P (0.67) N:P (0.72)	P (0.93) N:P (0.68)	P (0.88) N:P (0.66)	C (0.86) N (0.75) P (0.95) N:P (0.60)	
Soil (0–20 cm)	C:P (0.86) N:P (0.79)	C:P (0.73) N:P (0.79)	C:P (0.82) N:P (0.87)	C:P (0.78) N:P (0.85)	C:P (0.82) N:P (0.69)	C:P (0.86) N:P (0.69)	C:P (0.73) N:P (0.88)

^a Numbers in the parentheses are Pearson correlation coefficients (r) with $P < 0.05$

et al. 2004). It is generally accepted that plants growing in nutrient-rich environments produce a well-developed litter layer with relatively high C:P and N:P ratios (Aerts 1996; Killingbeck 1996). However, litter quality also declines and P concentrations were reduced because P becomes subject to leaching, and is eventually depleted from the ecosystem. In contrast, N is more biologically renewable (Wardle et al. 2004b). In our study, litter C, N, and P concentrations significantly decreased at the 37-year-old stand (Fig. 2a–c). Litter C:N:P has long been recognized as a useful indicator of the source of organic matter, its state of decomposition, and its potential contribution to soil fertility (Agren et al. 2013; Bui and Henderson 2013; Wardle et al. 2004b). Our results indicate that litter organic matter accumulation was faster than its decomposition leading to a net immobilization of nutrients in black locust plantations on the Loess Plateau.

At the global scale, the C:P and N:P ratios in litter are 2.36 and 1.63 times higher than those in tree leaves (Cleveland and Liptzin 2007). Furthermore, the litter N:P ratio increases during stand development (Wardle et al. 2004b). In our study, however, the leaves and litter had similar C:P and N:P ratios, and the litter C:P and N:P ratios

remained stable with increasing stand age. This result is supported by a previous study reporting that the N₂-fixing black locust tree species have high N retranslocation efficiency, so that a larger proportion of absorbed N is converted into plant biomass and less is recycled through litterfall (Townsend et al. 2007). Therefore, there were distinctly different C:N:P ratios in the leaves (465:11:1) and litter (486:16:1) in the study plantations when compared to the ratios in the leaves (1212:28:1) and litter (3007:45:1) of natural forests worldwide (McGroddy et al. 2004).

Although N is frequently proposed as the primary limiting nutrient in forests, P is arguably more limiting for forested ecosystems during the declining growth phase (Lambers et al. 2010; Wardle et al. 2004b). According to the model of soil development proposed by Walker and Syers (1976), the nutrient limitation changes over the growth of forest species in soil development, transitioning from N limitation in young sites to P limitation in old sites. This model was supported by results from chronosequences for several thousand years (Hayes et al. 2014; Parfitt et al. 2005; Perez et al. 2014; Reed et al. 2011). Previous studies indicated typically low levels of P present in soils across

the national and regional levels in China (Fan et al. 2015; Han et al. 2005; Huang et al. 2013). Soil C, N, and P concentrations are highest in 2-year-old Eucalyptus plantations in subtropical China, and then decline in the 4- and 6-year-old ones, indicating that P limitation increases with plantation age (Fan et al. 2015). On the Loess Plateau, soil C and N have increased while P has decreased since the farm land was abandoned (Jiao et al. 2013). Moreover, our study found that P was more limiting than N in the oldest sites of the 37-year-old stand during the observed period.

Understanding the causes and consequences of relative stoichiometric balances within and between plants and soils can prove to be valuable in the application to specific consumer/resource interactions or to the sum of processes occurring within an ecosystem (Agren and Weih 2012; Gusewell 2004; McGroddy et al. 2004). Many studies have demonstrated strong relationships among C, N, and P in soils and in plants (Agren 2008; Cleveland and Liptzin 2007; Liao et al. 2014; McGroddy et al. 2004; Yang and Luo 2011). A relatively high correlation between C and N concentrations, and weak correlations between C and P concentrations and between N and P concentrations have been reported in surface soil layers in China (Tian et al. 2010). Leaf N and P concentrations, as well as soil N and P patterns, were closely correlated in south-western Australia (Lambers et al. 2010). In this study, we only found a significantly positive correlation ($r = 0.98$) between soil C and N (Table 2). Soil C and N in terrestrial ecosystems may not directly regulate soil P, but soil P ultimately influences the amount of biologically active P available for plant productivity. Therefore, there is an indirect link between the P, C, and N in the soil (Cleveland and Liptzin 2007). Previous studies observed that the N:P ratios and N concentrations in interspecific plants were unrelated, while the N:P ratios and P concentrations were negatively correlated (Aerts 1996; Gusewell 2004; Killingbeck 1996). We found strong positive correlations among plant tissues for elemental P and the C:P and N:P ratios, but not between elemental P and N:P ratios in litter and other plant tissues (Table 2).

The C:N:P ratio in soil directly reflects soil fertility and indirectly serves as an indicator of plant nutritional status (Elser et al. 2010). A better understanding of element coupling in the above- and below-ground compartments is required to predict how nutrient cycles interact with C-cycling in diverse ecosystems (Hessen et al. 2004). A fundamental study found positive correlations between plant leaf and soil nutrients in 1900 plant species across China (Han et al. 2011). Correlations were found only between P concentrations in the 10–20 cm soil layer and in leaves of black locust plants on the Loess Plateau (Li et al. 2013). Another study noted a positive relationship between leaf N and soil N concentration in the organic horizon

layers in temperate rain forests along three New Zealand chronosequences (Parfitt et al. 2005). Regression analyses showed that soil N:P ratios were significantly correlated with leaf N:P ratios in subtropical Eucalyptus plantations (Fan et al. 2015). This is mainly due to the soil P supply and similar N and P retranslocation patterns (Fife et al. 2008; Hedin 2004). Positive relationships between soil C:N:P and leaf nutrient ratios in Australia's major native vegetation ecosystems have also been observed (Bui and Henderson 2013). In our study, there were strong positive correlations between soil and plant tissues for the C:P and N:P ratios. However, coupling relations between C, N, and P between soils and forest plants have not always been observed (Jobbágy and Jackson 2001; Ladanai et al. 2010; Yu et al. 2010).

Moreover, negative correlations between N:P and growth rate have been reported for *Betula pendula* and *Pinus contorta* (Agren 2004; Matzek and Vitousek 2009). Negative relationships between tree growth and N:P ratio, as well as positive relationships between understory biomass and N:P ratio, have been observed in plantations of subtropical China (Fan et al. 2015). This is mainly due to different nutrient acquisition patterns of understory and overstory plants; small plants maintain higher nutrient concentrations and lower N:P ratios than larger trees (Elser et al. 2010; Gusewell 2004). In addition, N:P ratios increase at low plant growth rates and then decrease at high growth rates (Agren 2004). Thus, interactions between leaf traits and soil nutrients, variations in climate, plant-type, and possibly species-level interactions may account for the regulation of foliar chemistry, which can in turn determine the thresholds in limitations of either N or P (Gusewell 2004; Reich and Oleksyn 2004; Richardson et al. 2008; Townsend et al. 2007).

In summary, C:N:P stoichiometry exerts a determining role in the functions and structure of ecosystems (Carnicer et al. 2015; Sardans et al. 2012). The available evidence highlights that multiple ecological processes have been largely impacted by increased N deposition rates and growing C:N:P imbalances (Pan et al. 2015; Wang and Moore 2014). In the next decades, ecosystem functions will simultaneously respond to increased C:N:P imbalances and global warming, changes in the geographic distribution of functional groups, invasive species, altered disturbance regimes and habitat destruction and alteration, among other drivers in terrestrial ecosystems (Carnicer et al. 2015; Sardans et al. 2012; Yue et al. 2017). Here, we only explored the coupling of plant and soil C:N:P stoichiometry in black locust plantations across age sequence on the Loess Plateau, China. However, the complexity factors controlling species-specific C:N:P stoichiometry in different functional groups still remain largely uncertain (Yue et al. 2017). Therefore, it is necessary to study further these

basic questions offer clues to understanding how nutrient limitation develops during ecosystem succession, what are the rates of limitation over time, how do species and ecosystems respond to nutrient limitation over succession time, and provide management practices for forest policy-makers to create sustainable forest ecosystem, particularly for the large-scale plantations on the Loess plateau, China.

Conclusions

Overall, the tree tissues, herbs, litter, and soils exhibited different nutrient stoichiometry patterns during stand development on the Loess Plateau. More specifically, P concentrations in plant tissues and litter significantly decreased at the mature stage (37-year-old stands). Consequently, the ratios of C:P and N:P increased in all components of the black locust plantations during stand development. Furthermore, strong linkages among ecological components of the black locust plantation were highlighted by the N:P ratio. These results strongly support the idea that elemental P serves as a controlling element and is the key interactive nutrient affecting biogeochemical cycles throughout the plant growth and development process. Thus, the age-related stoichiometric imbalances observed in the elemental supply and demand could be the driving limiting factor affecting the long-term development of the black locust plantations on the Loess Plateau. Therefore, to create sustainable forest ecosystem, plantation management practices should pay close attention to shift in plant community composition, and change in the stoichiometry of resource inputs to avoid long-term P depletion through multiple ecological mechanisms.

Author contribution statement YCa and YCh designed and carried out the experiments. YCa wrote the manuscript with contributions from YCh.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to declare.

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