Forest Ecosystems 10 (2023) 100123

Contents lists available at ScienceDirect

Forest Ecosystems

journal homepage: www.keaipublishing.com/cn/journals/forest-ecosystems

Ecological stoichiometric comparison of plant-litter-soil system in mixed-species and monoculture plantations of *Robinia pseudoacacia*, *Amygdalus davidiana*, and *Armeniaca sibirica* in the Loess Hilly Region of China

Senbao Lu [a,b], Yunming Chen [a,c,*], Jordi Sardans [d,e], Josep Peñuelas [d,e]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, 712100, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] Institute of Soil and Water Conservation, Northwest A&F University, Yangling, 712100, China
[d] CSIC, Global Ecology Unit CREAF-CSIC-UAB, Bellaterra, 08193, Catalonia, Spain
[e] CREAF, Cerdanyola del Vallès, 08193, Catalonia, Spain

ARTICLE INFO

Keywords:
Stoichiometry
Plant
Litter
Soil
Tree mixture
Loess Plateau

ABSTRACT

We examined how afforestation patterns impact carbon (C), nitrogen (N), and phosphorus (P) stoichiometry in the plant-litter-soil system. Plant leaf, branch, stem, and root, litter, and soil samples were collected from mixed-species plantations of *Robinia pseudoacacia* with *Amygdalus davidiana* (RPAD), *R. pseudoacacia* with *Armeniaca sibirica* (RPAS), and monocultures of *R. pseudoacacia* (RP), *A. davidiana* (AD), and *A. sibirica* (AS) in the Loess Hilly Region. The results showed that in mixed-species plantations, *R. pseudoacacia* had lower leaf N and P concentrations than in monocultures, while both *A. davidiana* and *A. sibirica* had higher leaf N and P concentrations. Soil P limited tree growth in both afforestation models. Mixing *R. pseudoacacia* with *A. davidiana* or *A. sibirica* reduced N-limitation during litter decomposition. Average soil total N and P concentrations were higher in RPAS than in RPAD, and both were higher than the corresponding monocultures. The average soil C:N ratio was the smallest in RPAS, while the average soil C:P ratio was larger in RPAS than in RP. A positive correlation between N and P concentrations, and between C:N and C:P ratios, was found in litter and all plant organs of mono- and mixed-stands. Alternatively, for N concentration and C:N ratio, the correlations between plant (i.e., leaf, branch, root) and litter and between plant and soil were inverse between plantation types. RPAD has an increased litter decomposition rate to release N and P, while RPAS has a faster rate of soil N mineralization. RPAD was the best plantation (mixed) to improve biogeochemical cycling, as soil nutrient restrictions, particularly for P-limitation, on trees growth were alleviated. This study thus provides insights into suitable tree selection and management by revealing C:N:P stoichiometry in the plant-litter-soil system under different afforestation patterns.

1. Introduction

Plantations in the Loess Hilly Region are divided into mixed-species and monoculture plantations. Mixed-species plantations consist of two or more species of trees. Generally, mixing suitable tree species can improve soil fertility, regulate microclimate, and reduce the occurrence and spread of pests, diseases, and fires (Camp, 1986; Coll et al., 2018). Previous studies have shown that mixed-species plantations promote nutrient circulation via canopy differentiation, root stratification, and the complementarity effect with respect to corresponding monocultures (Khanna et al., 2008). To preserve existing plantations and create new plantations, it is often desirable to convert monocultures into species-rich mixed plantations, as it is frequently observed that the stability and sustainability of mixed-species plantations is significantly higher than that of monocultures (Forrester et al., 2006). However, the sustainable development of mixed-species plantations depends not only on the individual characteristics of tree species (Wojciech et al., 2019), but also on site conditions, such as soil nutrient status (He and Dijkstra, 2014;

* Corresponding author. State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, 712100, China.
E-mail address: ymchen@ms.iswc.ac.cn (Y. Chen).

https://doi.org/10.1016/j.fecs.2023.100123
Received 12 January 2023; Received in revised form 5 June 2023; Accepted 5 June 2023

Sardans et al., 2017). Thus, it is difficult to predict the species compositions that would improve the stability of mixed-species plantation (Forrester et al., 2014).

Carbon (C), nitrogen (N), and phosphorus (P) stoichiometry focuses on the interactions and balance of chemical elements in the biogeochemical process (Sterner and Elser, 2002; Güsewell, 2004). It is also used to provide a scientific approach to study plant growth and nutrient limitations in a terrestrial ecosystem (Peñuelas and Baldocchi, 2019). Previous studies have analyzed the C:N:P stoichiometry of plants from a regional to global scale, in mixed-species plantations, to uncover nutrient cycling, limitations, and feedback of multiple tree species (Yang et al., 2018; Hou et al., 2018). Few studies have revealed the plant C:N:P stoichiometry discrepancy between mixed-species plantations and their corresponding monocultures. Existing studies have focused mainly on a few organs, such as the leaf (Jiao et al., 2022) and fine root (Wu et al., 2021b). Additionally, differences in C:N:P stoichiometry among tree species in different plantations can reflect the relationship of plant nutrient status with biogeochemical processes in the community (Dawud et al., 2016). Thus, C:N:P stoichiometry provides an opportunity to understand plant nutrient relationships in mixed-species plantations.

Soil is the primary source of nutrients for plant growth. Soil nutrient concentrations are affected by litter, organic matter, and soil microorganisms (Richard et al., 2013). Studies on mixed plantations of *Hippophae rhamnoides* reported a strong relationship between leaf and litter C:N:P stoichiometry, and the correlation between N and P concentrations was more significant among leaf, litter, and soil than those corresponding to C concentration (Wu et al., 2021b). Jiao et al. (2022) found that P concentration and C:P ratio were significantly correlated in litter and soil in mixed-species plantations. Chapin et al. (2011) found that 90% of the N and P elements released from litter into the soil were reabsorbed and utilized by plants. Pang et al. (2021) studied the C:N:P stoichiometry of secondary mixed forests in the Qinling Mountains and found a close relationship in the plant-litter-soil continuum. Thus, litter influences the chemical and physical properties of soil, and microbial activities through the release of organic carbon and nutrients, and can indirectly affect nutrient allocation to distinct organs via plant-soil nutrient cycling (Chapman and Newman, 2010; Yang et al., 2018).

The Loess Plateau is one of the most eroded loess regions in the world. To reduce water loss and soil erosion, the Chinese government implemented the "Grain to Green" project in the 1950's (Feng et al., 2016). To date, the area covered by plantation (7.5×10^4 ha) accounts for 60% of the total area (Liu et al., 2017). Due to its high growth rate and ability to improve soil nutrient conditions via N_2-fixing (Shan et al., 2002), *Robinia pseudoacacia* ($>7.0 \times 10^4$ ha) has become the most important introduced tree species for afforestation in this region (Cao and Chen, 2017). However, at half-maturity (19–25 years), the *R. pseudoacacia* community displayed growth slowdown and canopy wilting, implying reduced ecosystem services (Wei et al., 2018). *Amygdalus davidiana* and *Armeniaca sibirica*, two native tree species in the Loess Hilly Region, possess drought-resistant, well-developed roots and strong barberries. In addition, the kernel of *A. davidiana* is often made for decoration, while the pulp of *A. sibirica* is edible. To conserve soil and water and improve economic benefits, these two trees are widely cultivated with *R. pseudoacacia*. Previous studies primarily focused on the plant and soil C:N:P stoichiometry of *R. pseudoacacia* plantations (Cao and Chen, 2017; Zhang et al., 2019); however, the correlation of C:N:P stoichiometry among the plant-litter-soil system of monocultures and mixed-species plantations have been rarely evaluated. In this context, several studies have shown that soil and litter nutrient characteristics together influence the nutrient characteristics of plant (Chapin et al., 2011). The intrinsic connections between plant nutrients stoichiometry and the nutrient characteristics of litter can improve our understanding of plant nutrient limitations and ecosystem dynamics, according to stoichiometry theory. An overall view of plant stoichiometry shifts to environmental changes is necessary to discern the stoichiometric changes of the most plant organs as possible. For example, Gargallo-Garriga et al. (2014, 2015) observed that plants submitted to drought in a mesic European shrublands increased their foliar C:N and C:P ratios associated with a metabolic shift towards over production of C-rich secondary metabolites linked to anti-stress mechanisms, whereas decreased their root C:N, C:P, and N:P ratios linked to an increase of metabolism. Therefore, the analysis of stoichiometric changes in the distinct compartments of the plant-soil system is warranted to obtain an overall view of the plant-soil system response to environmental changes from a stoichiometric point of view and relate them to ecophysiological responses.

To address this knowledge gap, we investigated C:N:P stoichiometric of plant organ (i.e., leaf, branch, stem, root), litter, and soil samples in mixed-species and monoculture plantations of *R. pseudoacacia*, *A. davidiana*, and *A. sibirica* in the Loess Hilly Region. Our concrete aims were to understand: (1) whether mixed-species plantations changed the C:N:P stoichiometric of plant organs, litter, and soil compared to monocultures; (2) whether litter C:N:P correlated with the C:N:P stoichiometry of plant organs or soil; and (3) whether soil C:N:P correlated with the C:N:P ratio of other plant organs. We hypothesized that (1) the dynamic concentrations of C:N:P stoichiometry in the plant-litter-soil system may be influenced by different tree species, afforestation patterns, or the plant organ and soil layer used for analysis; (2) the correlations between C, N, and P concentrations and ratios would be all positive or negative in plant organs, litter, and soil of different plantations; and (3) the leaf, branch, and fine root composition of N and P would be derived from litter decomposition, while the C:N:P stoichiometry of stem may be more related to bulk soil.

2. Materials and methods

2.1. Site description

This study was performed in Ansai County (36°35′49″–36°35′56″ N, 109°13′18″–109°13′32″ E, with an average elevation of 1,450 m), located on the interior of the Loess Plateau (Fig. 1a). This region has a warm, temperate, semi-arid, continental monsoon climate, with an annual mean temperature of 8.8 °C. The maximum and minimum temperatures are 36.8 and −23.6 °C, respectively. The mean annual precipitation is 500 mm, with the rainy season (June–September) accounting for 72.9% of the total annual rainfall. Soils in this area are classified as Calcic Cambisols (FAO, 2020). Excessive logging and reclamation in the study area have resulted in less natural forest, and an increase in artificially created landscapes (Fu et al., 2017). Among local forest plantations, the primary tree species are *R. pseudoacacia*, *Pinus tabuliformis*, *Platycladus orientalis*, *Ulmus pumila*, *A. davidiana*, and *A. sibirica*. Shrub species include *H. rhamnoides* and *Caragana intermedia*. Herb species include *Medicago sativa*, *Herba artemisiae*, *Stipa bungeana*, *Bothriochloa ischaemum*, and *Artemisia gmelinii.*

2.2. Experimental design

Sampling sites were selected in mixed-species plantations that included mixed-species plantations of *R. pseudoacacia* with *A. davidiana* (RPAD), *R. pseudoacacia* with *A. sibirica* (RPAS) and in monocultures of *R. pseudoacacia* (RP), *A. davidiana* (AD), and *A. sibirica* (AS) at half-maturity (19–25 years) with normal growth and similar structures (Fig. 1b). In April 2021, three 20 m × 20 m plots (separated by more than 100 m) were established in each of five different plantations. Coordinate and elevation information for the sampling sites was marked as experimental plot sites using Ovey map (V.9.3.0., Beijing, China) (Table 1). Topographic factors (i.e., gradient, aspect) were determined with a hand-held GPS compass (JSD-X2., Beijing, China) (Table 1). Stand structures of all tree individuals in each plot were investigated (Table 2), including stand density (SD), crown area (CA), average tree height (AH), diameter at breast height (DBH), and height under branch (HB) (Table 2).

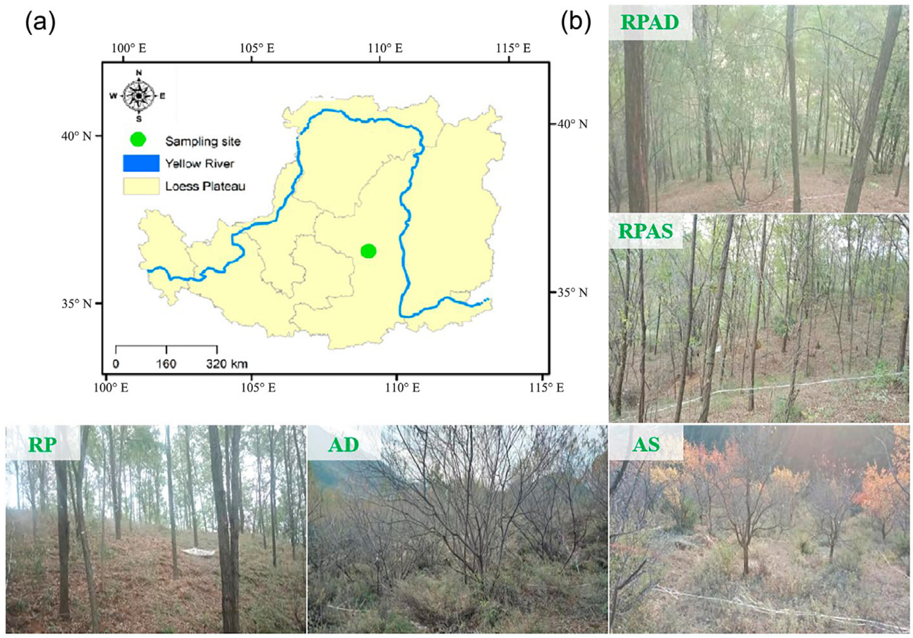

Fig. 1. Sample plot (**b**) is located on the Loess Plateau of China (**a**). RPAD: *R. pseudoacacia* and *A. davidiana* mixed plantation; RPAS: *R. pseudoacacia* and *A. sibirica* mixed plantation; RP: *R. pseudoacacia* monoculture; AD: *A. davidiana* monoculture; and AS: *A. sibirica* monoculture.

Table 1
Basic information for the sample sites and soil characteristics of mixed-species and monoculture plantations.

Afforestation pattern	Plantation type	Dominant species	No.	Longitude (N)	Latitude (E)	Altitude (m)	Gradient (°)	Aspect
Mixture	RPAD	*R. pseudoacacia* + *A. davidiana*	1	109°13′42.56″	36°35′52.03″	1189	20.4	Semi-sunny
			2	109°13′27.27″	36°35′29.35″	1191	29.2	Semi-sunny
			3	109°13′12.22″	36°35′30.82″	1199	22.6	Semi-sunny
	RPAS	*R. pseudoacacia* + *A. sibirica*	1	109°13′50.88″	36°35′21.10″	1195	22.3	Semi-sunny
			2	109°13′45.18″	36°35′34.17″	1142	23.9	Semi-sunny
			3	109°13′36.87″	36°35′50.74″	1203	20.4	Semi-sunny
Monoculture	RP	*R. pseudoacacia*	1	109°13′39.72″	36°35′50.85″	1190	21.4	Semi-sunny
			2	109°13′56.46″	36°35′24.88″	1212	21.7	Semi-sunny
			3	109°13′19.84″	36°35′29.70″	1180	14.0	Semi-sunny
	AD	*A. davidiana*	1	109°13′36.83″	36°35′50.32″	1173	28.8	Semi-sunny
			2	109°13′39.36″	36°35′47.72″	1157	25.0	Semi-sunny
			3	109°13′43.43″	36°35′49.19″	1160	21.0	Semi-sunny
	AS	*A. sibirica*	1	109°13′50.28″	36°35′57.59″	1160	24.0	Semi-sunny
			2	109°13′56.90″	36°35′48.96″	1222	18.6	Semi-sunny
			3	109°16′31.85″	36°36′40.02″	1224	17.3	Semi-sunny

Notes: RPAD, *R. pseudoacacia* and *A. davidiana*; RPAS, *R. pseudoacacia* and *A. sibirica*; RP, *R. pseudoacacia*; AD: *A. davidiana*; AS, *A. sibirica*.

Table 2
Descriptive statistics of tree growth and soil properties among mixed-species and monoculture plantations.

Afforestation pattern	Plantation type	Dominant species	SD (trees·ha^{-1})		CA (m^2)	AH (m)	DBH (cm)	HB (m)	BD (g·cm^{-3})	SWC (%)	EC (mS·cm^{-1})	pH
			Species	Total								
Mixture	RPAD	*R. pseudoacacia*	1542 ± 213 a	1891 ± 150 A	262.14 ± 46.86	8.37 ± 0.23 ab	9.35 ± 0.26	3.41 ± 0.19 ab	1.25 ± 0.05 A	11.13 ± 0.63 A	107.57 ± 0.98 A	8.58 ± 0.12
		A. davidiana	350 ± 63 b		92.67 ± 15.48 b	4.72 ± 0.29 a	6.01 ± 0.30	1.08 ± 0.08 a				
	RPAS	*R. pseudoacacia*	792 ± 122 b	1167 ± 101 B	215.86 ± 54.42	8.09 ± 0.50 b	9.02 ± 0.81	3.03 ± 0.24 b	1.25 ± 0.04 A	9.91 ± 0.08 B	107.33 ± 1.11 A	8.52 ± 0.06
		A. sibirica	375 ± 109 b		93.94 ± 14.21	5.96 ± 0.25 a	9.62 ± 0.41	0.57 ± 0.07 a				
Monoculture	RP	*R. pseudoacacia*	1075 ± 76 ab	1075 ± 76 B	190.04 ± 2.87	9.63 ± 0.39 a	11.67 ± 1.11	3.84 ± 0.23 a	1.24 ± 0.05 A	9.44 ± 0.06 B	105.50 ± 0.60 A	8.66 ± 0.01
	AD	*A. davidiana*	733 ± 33 a	733 ± 33 C	143.17 ± 5.47 a	3.58 ± 0.12 b	5.65 ± 0.64	0.37 ± 0.05 b	1.19 ± 0.05 B	10.54 ± 0.08 AB	82.30 ± 0.76 C	8.72 ± 0.03
	AS	*A. sibirica*	700 ± 58a	700 ± 58 C	126.66 ± 7.23	3.46 ± 0.05 b	10.09 ± 0.77	0.34 ± 0.04 b	1.27 ± 0.05 A	7.91 ± 0.19 C	87.95 ± 0.81 B	8.72 ± 0.03

Notes: RPAD, *R. pseudoacacia* and *A. davidiana*; RPAS, *R. pseudoacacia* and *A. sibirica*; RP, *R. pseudoacacia*; AD: *A. davidiana*; AS, *A. sibirica*. SD, stand density; CA, crown area; AH, average tree height; DBH, diameter at breast height; HB, height under branch; BD, bulk density; SWC, soil water content; EC, electric conductivity. Different uppercase letters above the bars indicate significant differences among different plantation types, while different lowercase letters indicate significant differences among different plantation types for the same tree species ($P < 0.05$).

2.3. Sample collection

In August 2021, five target trees with similar height and DBH were selected for sampling in each plot. Three branches with the same diameter (5 mm) were collected from the upper, middle, and lower canopies in order to collect intact mature leaf samples without pest or disease damage. To reduce the effects of asymmetric tree trunk growth, stem samples were taken from both directions (east-west and north-south) of the trunk using growth cones (diameter = 0.5 cm). Since no suitable allometric equation can simultaneously calculate the biomass of all three trees, a universal coefficient of 0.5 was used to represent carbon storage in various organs of different tree species in mixed-species plantations (Zeng et al., 2018). A biomass weighted average method was used to calculate the C, N, and P concentrations of each organ in mixed-species plantations, with the calculation formula as follows (Liu et al., 2019):

$$E_{org} = \frac{\sum E_i \times B_i}{\sum B_{org}} \tag{1}$$

where E_{org} is organ C, N, and P concentrations of mixed-species plantations; E_i and B_i are the organ C, N, and P concentrations and biomass of each tree species, respectively; and B_{org} is the organ biomass of all tree species in the mixed-species plantations.

Nine sample points were established along an S-shape in each plot, and a root drill (diameter = 9 cm) was used to obtain fine root (diameter <2 mm) samples at 0–20, 20–40, and 40–60 cm. Soil samples were collected simultaneously. In addition, single soil samples at 0–20 cm depth profile were collected from each plot using 100 cm^3 ring knife. To avoid the effect of fine root and litter on the sample, the soil organic layer and debris was removed prior to sampling. In mid-November 2021, freshly fallen and not yet decomposed leaf litter was collected. Nutrient composition from a soil depth of 0–60 cm was calculated using the following equation:

$$E_{soil} = \frac{\sum E_k \times BD_k \times T_k}{\sum BD_k \times T_k} \tag{2}$$

where E_{soil} is C, N, and P concentrations in 0–60 cm soil layer, E_k is C, N, and P concentrations in k soil layer, BD_k is bulk density in k soil layer, and T_k is depth in k soil layer.

2.4. Laboratory analyses

Plant and litter samples were oven-dried at 60 °C for 48 h to constant weight (Yang et al., 2018), and subsequently ground up and sieved (diameter = 0.25 mm). Soil samples were air-dried and passed through 2-mm mesh sieves to remove large roots and stones. They were then ground and sieved through 0.15-mm mesh to measure soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) (Pang et al., 2021). The C concentration of plant, litter, and soil (organic carbon) samples was determined via oil bath $K_2Cr_2O_7$ titration (Nelson and Sommers, 1982). Organ, litter, and soil samples were digested with H_2SO_4–H_2O_2 and H_2SO_4–$HClO_4$. N and P concentrations were then determined using the semi-automatic Kjeldahl method (Bremner and Mulvaney, 1982) and colorimetric method (Murphy and Riley, 1962), respectively. Soil bulk density (BD) and soil water content (SWC) were measured using the cutting ring method (Bao, 2000), soil pH was determined using an automatic acid–base titrator (PB-10 standard pH meter; Sartorius, Göttingen, Germany) with a water:soil ratio of 2.5:1, and electric conductivity (EC) was determined using a conductivity meter (DDSJ-308F, INESA Scientific InstrumentCo., Ltd, Shanghai, China). Notes: roots (and all the organs) of each species were analyzed as separate samples and not mixed samples of the two species previous to analyses to determine C, N and P.

2.5. Statistical analysis

All data was subject to the one-sample Kolmogorov-Smirnov test, which subsequently met the requirements for normal distribution. A one-way ANOVA and Duncan test were used to compare the discrepancy of C:N:P stoichiometry in plant, litter, and soil among mixed-species and monoculture plantations. A *t*-test was used to compare the discrepancies between C:N:P stoichiometry for the same species in different plantations. A principal component analysis (PCA) and Pearson correlation coefficient were used to determine the correlations within the C:N:P stoichiometry in the plant-litter-soil system. A linear mixed model ANOVA was used to determine the effects of tree species (TS) and afforestation pattern (mixed versus mono, AP) on the C:N:P stoichiometry of litter, as well as to determine the effects of the average soil, TS, AP, and plant organ (PO) on the plant C:N:P stoichiometry. The effects of TS, AP, and soil layer (SL) on the soil C:N:P stoichiometry was also assessed. Linear regression analysis was used to determine the relationships of C:N:P stoichiometry among plant, litter, and soil, as well as between plants and their contributing factors, i.e., litter or soil. Variation partitioning analysis (VPA) was used to determine the contribution of litter and soil to the plant C:N:P stoichiometry in different plantations. SPSS 18.0 (IBM Inc., New York, USA) was used to analyze the data. VPA and PCA were performed using Canoco 5 (Ter Braak and Smilauer, 2012). Figures were created using Origin 2017 software (Originlab Inc., USA). The significance level was set at $P < 0.05$.

3. Results

3.1. Plant growth and soil properties

As shown in Table 2, the afforestation pattern had no significant effect on CA, DBH, or pH ($P > 0.05$). Compared to monocultures, SD was smaller, while AH, and HB were larger for *A. davidiana* and *A. sibirica* in mixed plantations ($P < 0.05$); SD and AH did not change significantly for *R. pseudoacacia* in mixed plantations ($P > 0.05$), while HB was larger for *R. pseudoacacia* in RPAD ($P < 0.05$). Similarly, total SD was larger ($P < 0.05$), while AH and HB did not change significantly for *R. pseudoacacia* in RPAD than in RPAS ($P > 0.05$). In addition, SWC was larger ($P < 0.05$), while BD and EC did not change significantly in RPAD than in RPAS ($P > 0.05$).

3.2. Plant C:N:P stoichiometry

Fine root N and P concentrations of *R. pseudoacacia*, leaf and branch N and P concentrations of *A. davidiana* were higher in RPAD than in RP and AD, leaf N concentration, root P concentration of *R. pseudoacacia*, leaf and branch N concentrations, stem N concentration of *A. sibirica* were higher in RPAS than in RP and AS (Table 3). In addition, *R. pseudoacacia* in RPAD had higher branch and stem C, N and P concentrations than *R. pseudoacacia* in RPAS, while leaf and root C, N and P concentrations were in the other way around.

At the same time, C:P ratio of stem and branch, N:P ratio of fine root of *R. pseudoacacia*, N:P ratio of leaf and branch, C:P and N:P ratios of stem and root of *A. davidiana* were higher in RPAD than in RP and AD, branch, and stem C:N ratios of *R. pseudoacacia*, leaf and branch N:P ratios, stem N:P ratio of *A. sibirica* were higher in RPAS than in RP and AS (Table 4). In addition, *R. pseudoacacia* in RPAD had lower C:N and C:P ratios of leaf, branch, stem and root than *R. pseudoacacia* in RPAS, while the N:P ratio of leaf, branch, stem, and root was in other way around.

Several factors were found to have significant effects on plant C:N:P stoichiometry, including tree species, plant organ interactions, and interaction of tree species, plant organ, and afforestation pattern ($P < 0.05$, Table S1). Tree species, afforestation pattern, and plant organ interactions had significant effects on plant C concentration and C:P ratio ($P < 0.01$, Table S1).

Table 3
Characteristics of the C, N and P concentrations in plant (leaf, branch, stem, and fine root) among mixed-species and monoculture plantations. Data are means ± SE.

Element and their ratios	Plant organ	Pure plantation			Mixed plantation			
		R. pseudoacacia	*A. davidiana*	*A. Sibirica*	*R. pseudoacacia* + *A. davidiana*		*R. pseudoacacia* + *A. Sibirica*	
C	Leaf	470.00 ± 0.46 Db	481.60 ± 0.94 Ba	477.16 ± 0.75 Ca	455.54 ± 1.94 Dc	456.90 ± 0.90 Eb	489.42 ± 1.09 Ba	446.41 ± 0.33 Eb
	Branch	482.58 ± 1.23 Ca	463.64 ± 1.67 Db	462.47 ± 0.54 Ea	483.88 ± 2.63 Ca	496.42 ± 1.21 Ba	470.07 ± 0.90 Db	457.03 ± 1.07 Db
	Stem	492.64 ± 1.81 Ba	471.88 ± 1.06 Cb	503.30 ± 0.47 Ba	492.95 ± 2.64 Ba	481.11 ± 0.52 Da	477.22 ± 0.61 Cb	482.83 ± 0.72 Cb
	Root	510.12 ± 3.47 Aa	502.23 ± 1.41 Aa	546.47 ± 0.84 Aa	505.09 ± 1.56 Aa	507.90 ± 1.11 Aa	508.54 ± 1.39 Aa	534.94 ± 0.96 Ab
N	Leaf	28.21 ± 0.12 Aa	17.40 ± 0.37 Ab	13.10 ± 0.07 Ab	27.76 ± 0.23 Ba	25.45 ± 0.15 Aa	26.56 ± 0.20 Ab	16.73 ± 0.26 Aa
	Branch	10.11 ± 0.15 Da	6.56 ± 0.08 Db	4.50 ± 0.06 Bb	10.40 ± 0.29 Da	7.38 ± 0.19 Da	8.52 ± 0.27 Db	5.22 ± 0.13 Da
	Stem	3.35 ± 0.12 Ea	3.52 ± 0.09 Ea	2.51 ± 0.05 Ea	3.56 ± 0.21 Ea	2.29 ± 0.07 Eb	2.18 ± 0.10 Eb	1.88 ± 0.03 Eb
	Root	18.54 ± 0.13 Bc	7.52 ± 0.08 Cb	4.11 ± 0.09 Cb	29.93 ± 0.27 Aa	9.55 ± 0.24 Ca	20.14 ± 0.47 Bb	6.37 ± 0.07 Ca
P	Leaf	1.21 ± 0.04 Aa	0.95 ± 0.06 Ab	0.84 ± 0.01 Ab	0.95 ± 0.05 Ab	1.15 ± 0.02 Aa	1.19 ± 0.04 Aa	1.01 ± 0.03 Aa
	Branch	0.43 ± 0.01 Bc	0.46 ± 0.01 Cb	0.37 ± 0.00 Ba	0.52 ± 0.00 Ba	0.50 ± 0.01 Ca	0.47 ± 0.01 Bb	0.34 ± 0.03 Ca
	Stem	0.13 ± 0.02 Da	0.21 ± 0.01 Da	0.11 ± 0.01 Ea	0.11 ± 0.01 Cab	0.09 ± 0.00 Eb	0.09 ± 0.01 Db	0.10 ± 0.01 Db
	Root	0.39 ± 0.01 BCc	0.39 ± 0.02 Ca	0.21 ± 0.01 Db	0.56 ± 0.01 Ba	0.40 ± 0.02 Da	0.47 ± 0.01 Bb	0.39 ± 0.01 BCa

Notes: Different uppercase letters above the bars indicate significant differences among different plant organ for the same plantation type, while different lowercase letters indicate significant differences among different plantation type for the same plant organ ($P < 0.05$).

Table 4
Characteristics of the C:N, C:P, and N:P ratios in plant (leaf, branch, stem, and fine root) among mixed-species and monoculture plantations. Data are means ± SE.

Element and their ratios	Plant organ	Pure plantation			Mixed plantation			
		R. pseudoacacia	*A. davidiana*	*A. Sibirica*	*R. pseudoacacia* + *A.davidiana*		*R. pseudoacacia* + *A. Sibirica*	
C:N	Leaf	16.66 ± 0.06 Db	27.71 ± 0.55 Da	36.42 ± 0.14 Ea	16.41 ± 0.08 Cb	18.11 ± 0.07 Eb	18.43 ± 0.10 Ca	26.70 ± 0.40 Db
	Branch	47.74 ± 0.60 Bb	70.68 ± 0.62 Ba	102.90 ± 1.32 Da	46.57 ± 1.03 Bb	67.33 ± 1.61 Ba	55.28 ± 1.63 Ba	87.61 ± 1.93 Bb
	Stem	147.59 ± 4.86 Ab	134.05 ± 2.96 Ab	200.37 ± 4.04 Ab	139.38 ± 8.06 Ab	210.31 ± 6.51 Aa	219.73 ± 9.56 Aa	256.62 ± 3.77 Aa
	Root	27.51 ± 0.00 Ca	66.82 ± 0.53 Ba	133.10 ± 2.61 Ca	16.88 ± 0.10 Cc	53.25 ± 1.23 Cb	25.27 ± 0.54 Cb	83.99 ± 0.83 Bb
C:P	Leaf	390.55 ± 13.06 Cb	508.36 ± 29.70 Da	567.26 ± 9.12 Ea	480.04 ± 24.11 Ca	398.01 ± 5.94 Db	410.86 ± 11.08 Cb	443.40 ± 12.28 Cb
	Branch	1130.48 ± 35.54 Ba	1010.17 ± 9.65 Ca	1251.10 ± 12.44 Da	936.14 ± 3.35 Bc	997.25 ± 15.36 Bb	1008.09 ± 15.94 Bb	1360.52 ± 126.58 Ba
	Stem	3915.18 ± 487.00 Ab	2304.35 ± 68.16 Ab	4672.95 ± 242.43 Aa	4515.97 ± 207.46 Aab	5623.78 ± 177.36 Aa	5648.60 ± 407.44 Aa	4967.57 ± 297.64 Aa
	Root	1323.97 ± 32.51 Ba	1280.19 ± 68.21 Ba	2656.50 ± 92.46 Ba	896.64 ± 16.22 Bc	1262.79 ± 67.92 Ba	1095.35 ± 29.62 Bb	1385.64 ± 36.80 Bb
N:P	Leaf	23.44 ± 0.72 Bb	18.32 ± 0.74 ABb	15.57 ± 0.19 Cb	29.25 ± 1.38 Ca	21.98 ± 0.25 Ba	22.29 ± 0.49 Db	16.60 ± 0.28 Ba
	Branch	23.67 ± 0.59 Ba	14.29 ± 0.15 Ca	12.16 ± 0.04 Db	20.12 ± 0.46 Db	14.82 ± 0.21 Da	18.25 ± 0.31 Ec	15.48 ± 1.11 Ba
	Stem	26.38 ± 2.40 Bab	17.19 ± 0.41 Bb	23.30 ± 0.81 Aa	32.51 ± 1.61 Ca	26.74 ± 0.24 Aa	25.66 ± 1.06 Cb	19.34 ± 0.93 Ab
	Root	48.12 ± 1.17Ab	19.15 ± 0.88 Ab	19.95 ± 0.32 Ba	53.12 ± 0.68 Aa	23.69 ± 0.90 Ba	43.33 ± 0.37 Ac	16.49 ± 0.28 Bb

Notes: Different uppercase letters above the bars indicate significant differences among different plant organ for the same plantation type, while different lowercase letters indicate significant differences among different plantation type for the same plant organ ($P < 0.05$).

3.3. Litter and soil C:N:P stoichiometry

Litter C concentration and C:P ratio was smaller in RPAD than in RP and AD (Fig. 2a, e), while P concentration was larger (Fig. 2c), with similar findings in RPAS compared to RP and AS. Litter N:P ratio was measured in both RPAD and RPAS between their corresponding monocultures (Fig. 2f). Litter N and P concentrations and N:P ratio was higher in RPAD than in RPAS (Fig. 2b, c, f), while C concentration, C:N, and C:P ratios were smaller (Fig. 2a, d, e). The interaction between tree species and afforestation pattern had a significant effect on the litter C:N:P stoichiometry ($P < 0.01$, Table S2). Tree species, afforestation pattern, and their interaction had significant effects on the litter N and P concentrations, C:N, and C:P ratios ($P < 0.01$, Table S2).

Although soil nutrient "surface aggregation", soil C:N:P stoichiometry decreased with soil depth (Fig. 3). Under the topsoil, soil nutrient concentrations were larger in RPAD than in RP and AD, and in RPAS than in RP and AS (Fig. 3a–c); soil C:N ratios were smaller in RPAD than in RP and AD (Fig. 3d), while C:P and N:P ratios were larger than in RP and AD (Fig. 3e and f); C:N, C:P and N:P ratios in RPAS were between RP and AS (Fig. 3d–f). In addition, shallow SOC concentration, soil C:N, and C:P ratios were higher in RPAD than in RPAS (Fig. 3a, d–e), while shallow soil TN and TP concentrations and N:P ratios were smaller (Fig. 3b–c, f). Average SOC, soil TN, and TP concentrations were larger in RPAD than in RP and AD, and in RPAS than in RP and AS (Fig. 3a–c). In addition, the average SOC concentration, soil C:N, and C:P ratios was higher in RPAD than in RPAS (Fig. 3a, d–e), while shallow soil TN and TP concentrations and N:P ratio were smaller (Fig. 3b–c, f).

Afforestation pattern and soil layer had significant effects on the SOC, soil TN, and TP concentrations and C:N ratio ($P < 0.001$), though their interactions had inconsistent effects (Table S3). The average SOC and soil TN concentrations and N:P ratio were larger in RPAD than in RP and AD, as well as being larger in RPAS compared to RP and AS (Fig. 3a–b, f). Afforestation pattern had a significant effect on the average SOC and soil TN concentrations and N:P ratio ($P < 0.01$, Table S4).

3.4. Relationship of C:N:P stoichiometry within the plant, litter, and soil

In all plants structures (i.e., leaf, branch, stem, fine root), N and P

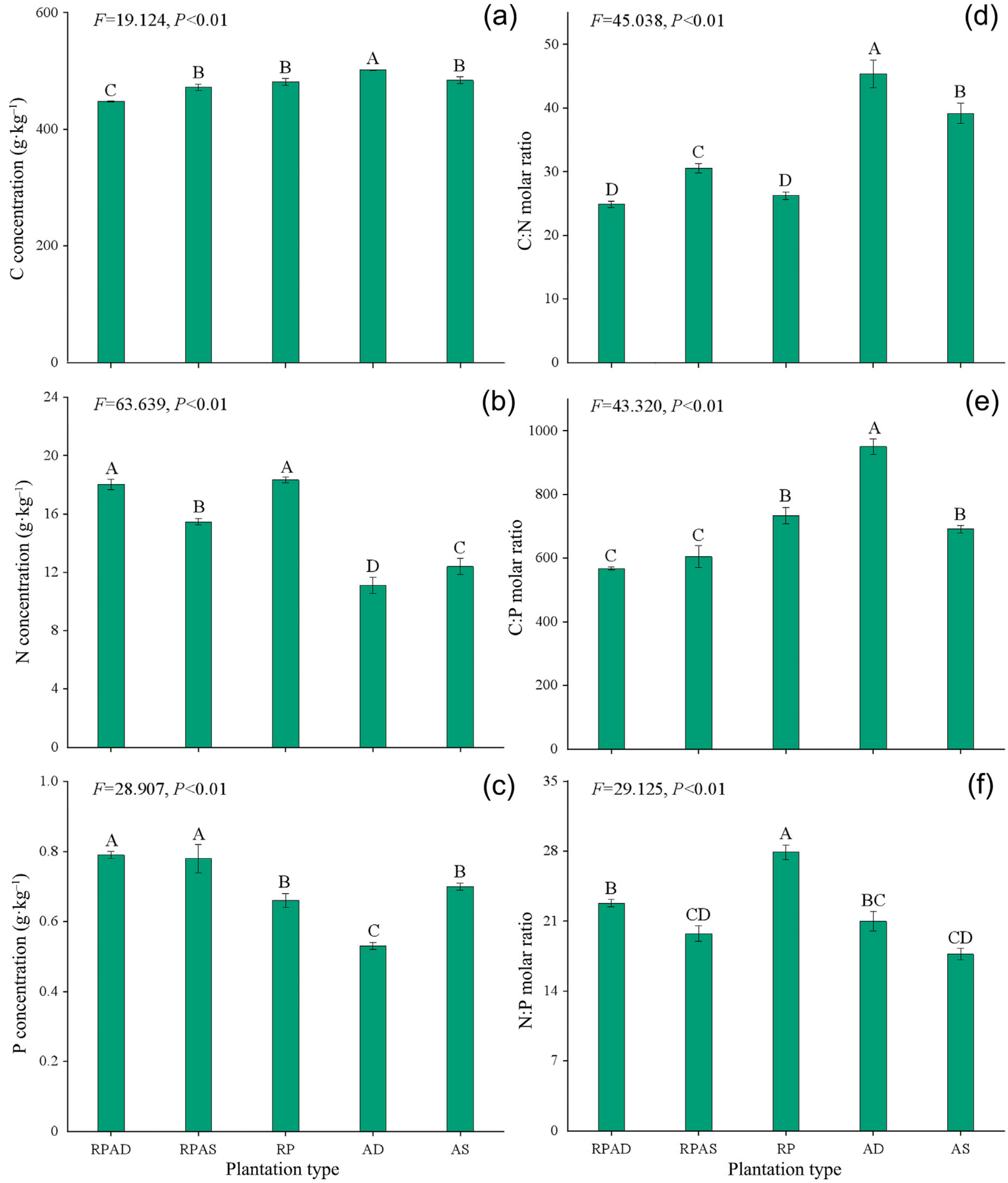

Fig. 2. Litter C, N, and P concentration (**a**, **b**, **c**) and stoichiometric ratio (**d**, **e**, **f**) in mixed-species and monoculture plantations. Data are means ± SE; different uppercase letters above the bars indicate significant differences among different plantations.

concentrations were positively correlated ($P < 0.05$, Fig. 4c), in addition to C:N and C:P ratios ($P < 0.05$, Fig. 4d). In litter and average soil, C and N concentrations were positively correlated ($P < 0.01$, Fig. 4a), as well as C:P and N:P ratios ($P < 0.01$, Fig. 4f).

A positive correlation was observed between litter N and P concentrations ($P < 0.05$, Fig. 5a and b), as well as between SOC and soil TN concentrations ($P < 0.05$, Fig. 5a and b), in RPAD and RPAS. Positive correlations were also found between plant (leaf, root) and soil P concentrations ($P < 0.01$, Fig. 5a and b) in RP, stem N and P concentrations ($P < 0.001$, Fig. 5a and b), leaf and litter C concentrations ($P < 0.001$, Fig. 5a and b) in AD, and plant (stem, root) and soil C concentrations ($P < 0.01$, Fig. 5a and b) in AS. Similarly, positive correlations were observed between stem and soil N:P ratio ($P < 0.01$, Fig. 5c and d) in RPAD and RPAS, plant (leaf, branch, and root) and litter N:P ratio ($P < 0.01$, Fig. 5c and d) in RP, litter C:P and C:N ratios ($P < 0.001$, Fig. 5c and d) in AD, and plant (leaf, branch, and root) and soil C:N and C:P ratios ($P < 0.05$, Fig. 5c and d) in AS.

3.5. *Effect of litter and soil on the plant C:N:P stoichiometry*

Litter N concentration exhibited a positive correlation with leaf, branch, and root N concentration ($P < 0.01$, Fig. 6b) in both mixed-species and monoculture plantations. Litter C:N and N:P ratios showed a positive correlation with root C:N and N:P ratios ($P < 0.05$, Fig. 6d, f),

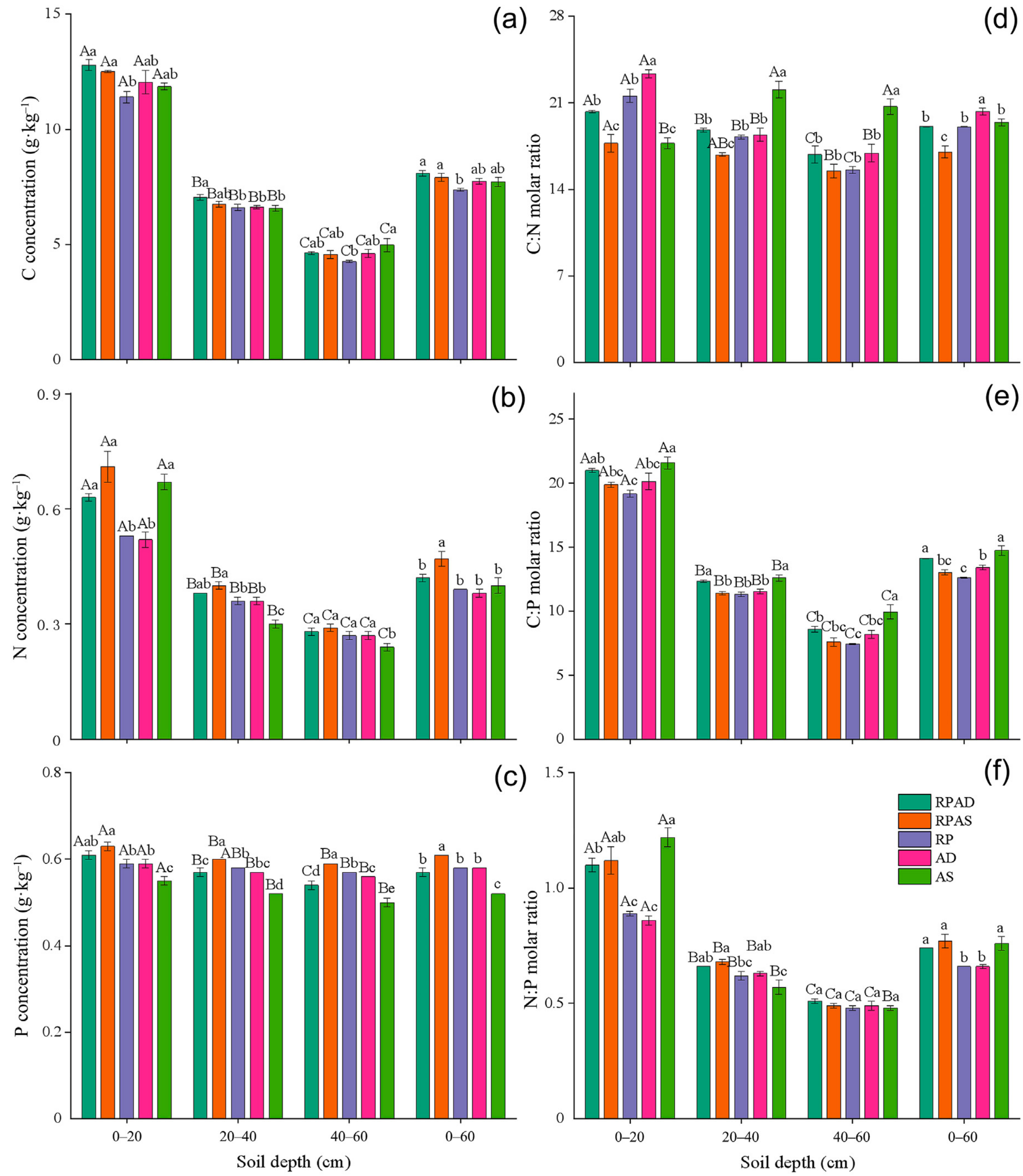

Fig. 3. The vertical distribution and the average soil C, N, and P concentration (**a**, **b**, **c**) and stoichiometric ratio (**d**, **e**, **f**) in mixed-species and monoculture plantations. Data are means ± SE; different uppercase letters above the bars indicate significant differences among different soil layers in the same plantation; different lowercase letters above the bars indicate significant differences among different plantations at the same soil layer.

while litter C concentration displayed a positive correlation with leaf C concentration ($P < 0.01$, Fig. 6a). Furthermore, litter C and P concentrations exhibited negative correlations with branch C concentration ($P < 0.01$, Fig. 6a) and stem P concentration ($P < 0.01$, Fig. 6c), respectively. Litter C:P ratio was negatively related to stem C:P ratio ($P < 0.01$, Fig. 6e). Soil TP concentration showed a positive correlation with leaf and root P concentration ($P < 0.01$, Fig. 7b), whereas soil C:P ratio displayed a positive relationship with leaf and root C:P ratio ($P < 0.05$, Fig. 7d). Furthermore, soil TN concentration and C:N ratio exhibited negative correlations with stem N concentration ($P < 0.05$, Fig. 7a) and C:N ratio ($P < 0.01$, Fig. 7c). Variance partitioning analysis revealed that litter demonstrated strong associations with leaf and branch C:N:P stoichiometry, as well as with stem and root stoichiometric ratios, while soil exhibited the strongest association with stem and root nutrient contents (Table 5).

4. Discussion

4.1. Comparison of C:N:P stoichiometry in plant, litter, and soil among mixed-species and monoculture plantations

4.1.1. Comparison of plant C:N:P stoichiometry among mixed-species and monoculture plantations

Wright et al. (2004) reported that tree species allocated the most

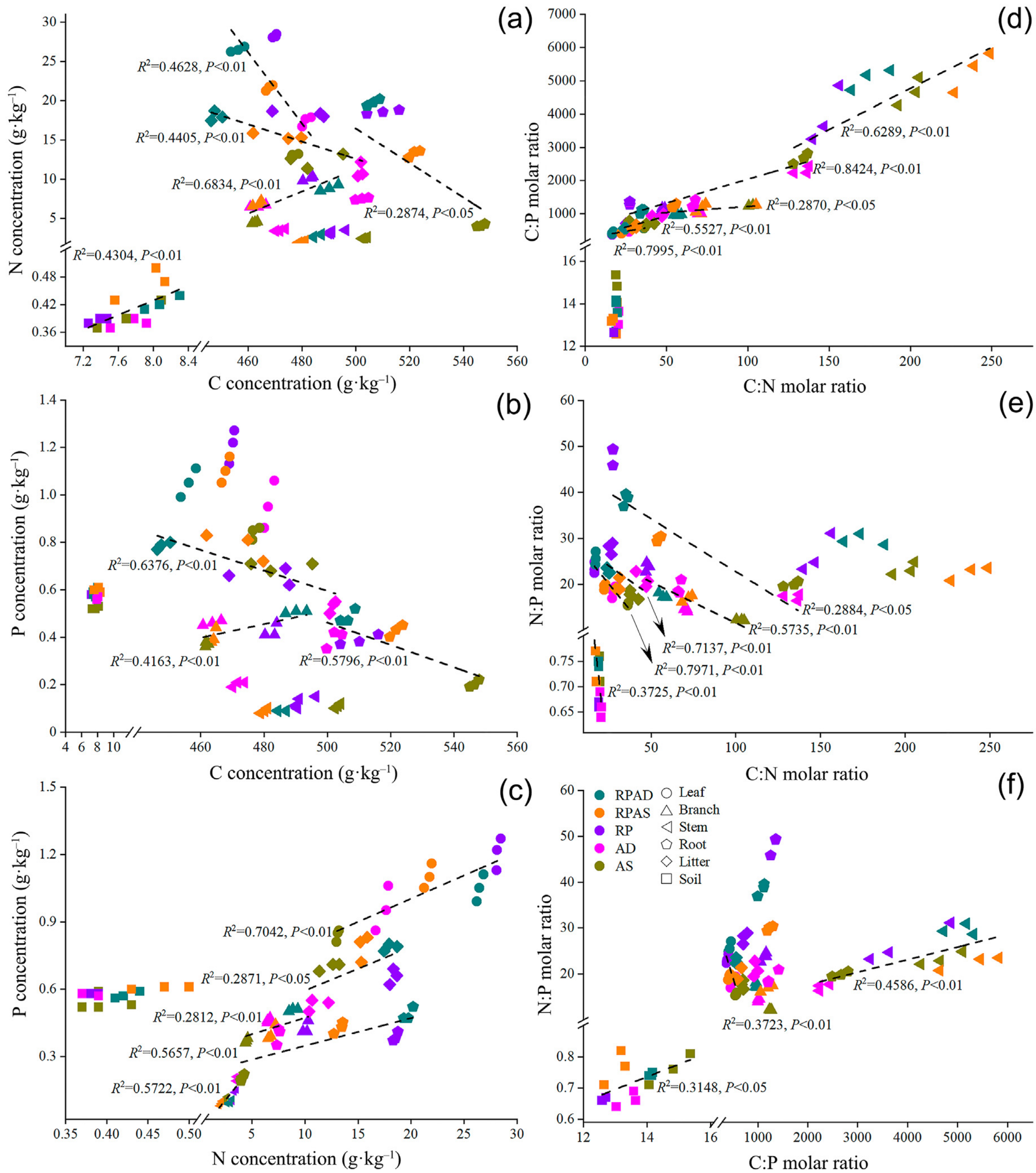

Fig. 4. Relationships between C and N concentrations (**a**), C and P concentrations (**b**), N and P concentrations (**c**), C:N and C:P ratios (**d**), C:N and N:P ratios (**e**), and C:P and N:P ratios (**f**) in the plant (leaf, branch, stem, fine root), litter, and average soil of different plantations.

nitrogen and phosphorus to their leaves, which is consistent with our results (Table 3). This may be because fast-growing tissues, such as the leaf, require more nitrogen and phosphorus for protein synthesis, resulting in lower C:N and C:P ratios (Table 4) (Yuan et al., 2011), and leaves must sustain all photosynthesis production to provide sources for all plant growth (Wang et al., 2015). Moreover, tree species are affected by their respective tissue structures and functional differentiation, leading to unique nutrient requirements (Sardans et al., 2017). Consistent with hypothesis 1, higher N concentrations were observed in leaves of *A. davidiana* in RPAD than *A. sibirica* in RPAS, and in *R. pseudoacacia* growing in RPAD than growing in RPAS (Table 3). A plausible explanation is that there is a segregation of species niche leading to differences in dynamic nutrients use efficiencies and a corresponding dilution effect in leaves (Bauhus et al., 2004).

Leaf N:P ratio is often used as an indicator to quantify the limiting nutrient in soil (Güsewell, 2004; Han et al., 2013). In our study, the growth of *R. pseudoacacia* in different plantations was limited by soil P (Table 4), which is consistent with previous research (Cao and Chen, 2017; Wu et al., 2021a; Jiao et al., 2022). Zhao et al. (1995) reported that leguminous plants need to consume a large amount of phosphorus in the nodulation process, and their roots secrete organic acids to activate insoluble phosphorus, thereby promoting plant uptake of phosphorus from the soil. Besides, mixed stands of *A. davidiana* in RPAD was no longer limited by soil N, while *A. sibirica* in RPAS was still limited by soil N (Table 4). Therefore, the ability of N_2-fixing bacteria to alleviate nitrogen limitation of symbiotic species may depend on the nitrogen requirements of the latter (Reich et al., 1992; Forrester, 2014; Yin et al., 2021).

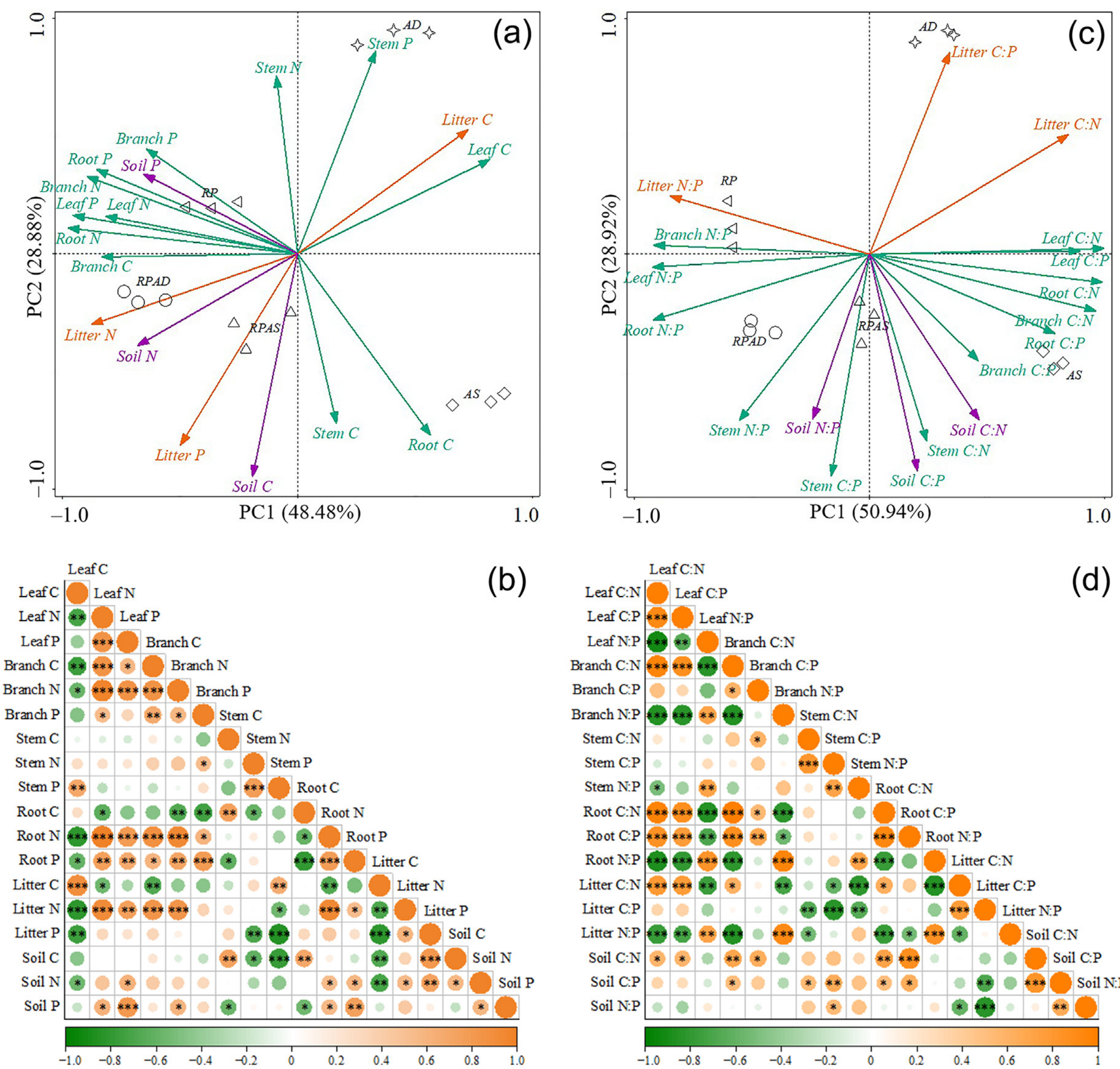

Fig. 5. Relationships among C, N, and P concentrations (**a**, **b**) and stoichiometric ratios (**c**, **d**) in the plant-litter-soil system of different plantations.

4.1.2. Comparison of litter C:N:P stoichiometry among mixed-species and monoculture plantations

Litter plays a major role in nutrient cycling between plants and soil, where organic matter is broken down into small inorganic molecules by microbes. Lower C:N and C:P ratios were beneficial to microbial activity, promoting faster rates of litter decomposition and nutrient release (Sariyildiz and Anderson, 2003; Manzoni et al., 2010). Based on this information, our data suggest that mixed stands of *A. davidiana* with *R. pseudoacacia* have increased litter decomposition rates compared with corresponding monocultures (Fig. 2d and e) (Farooq et al., 2022). This is consistent with the observation that shallow soil nutrient concentrations in mixed-species plantations were higher than in monocultures (Lü et al., 2012). However, the values of litter C:N ratio in mixed stands of *A. sibirica* and *R. pseudoacacia* were between the litter C:N ratio of their respective monocultures (Fig. 2d), suggests a relatively stable microbial abundance and diversity. Consistent with previous studies, senesced leaves with greater lignification from *A. sibirica* in RPAS are difficult to break down for microorganisms (Townsend et al., 2007; Laclau et al., 2010).

The decomposition of litter is limited by nutrients, which in the case of nitrogen and phosphorus can be reflected by the N:P ratio (Güsewell and Verhoeven, 2006). The thresholds of litter N- and P-limitation occur at 22 and 25 $g \cdot kg^{-1}$, respectively (Güsewell and Freeman, 2005). Compared to corresponding monocultures, mixes of *R. pseudoacacia* with *A. davidiana*, or *A. sibirica*, were found to alleviate N-limitation during litter decomposition (Fig. 2d), indicating that mixes of N_2-fixing and symbiotic tree species can improve nitrogen retranslocation efficiency (Townsend et al., 2007). In addition, the increased of litter decomposition rate was contributed to the N transfer rate (Britton et al., 2018).

4.1.3. Comparison of soil C:N:P stoichiometry among mixed-species and monoculture plantations

Species diversity of plantation can influence the released of litter products and root exudates into soil (Chapman and Newman, 2010; Zhang et al., 2022). Moreover, in mixed stands, different species habitually do not exploit exactly the same soil layers and thus this makes possible a better exploitation of soil resources and at some extent avoid direct competition (Forrester et al., 2006; Khanna et al., 2008). Besides, plantations with complex canopies structure are conductive to intercepting rainfall, resulting in reduced soil nutrients carried by spatter erosion and leaching (Eviner and Chapin, 2002). For these reasons, average soil TN and TP concentrations were larger in RPAS than that in RPAD, and larger in mixed-species plantations than in monocultures (Fig. 3b and c) (Lü et al., 2012). Whereas similar litter P concentrations in mixed-species plantations indicate that increased shallow soil TP concentrations were not just caused by litter (Fig. 2c, Fig. 3c). Different

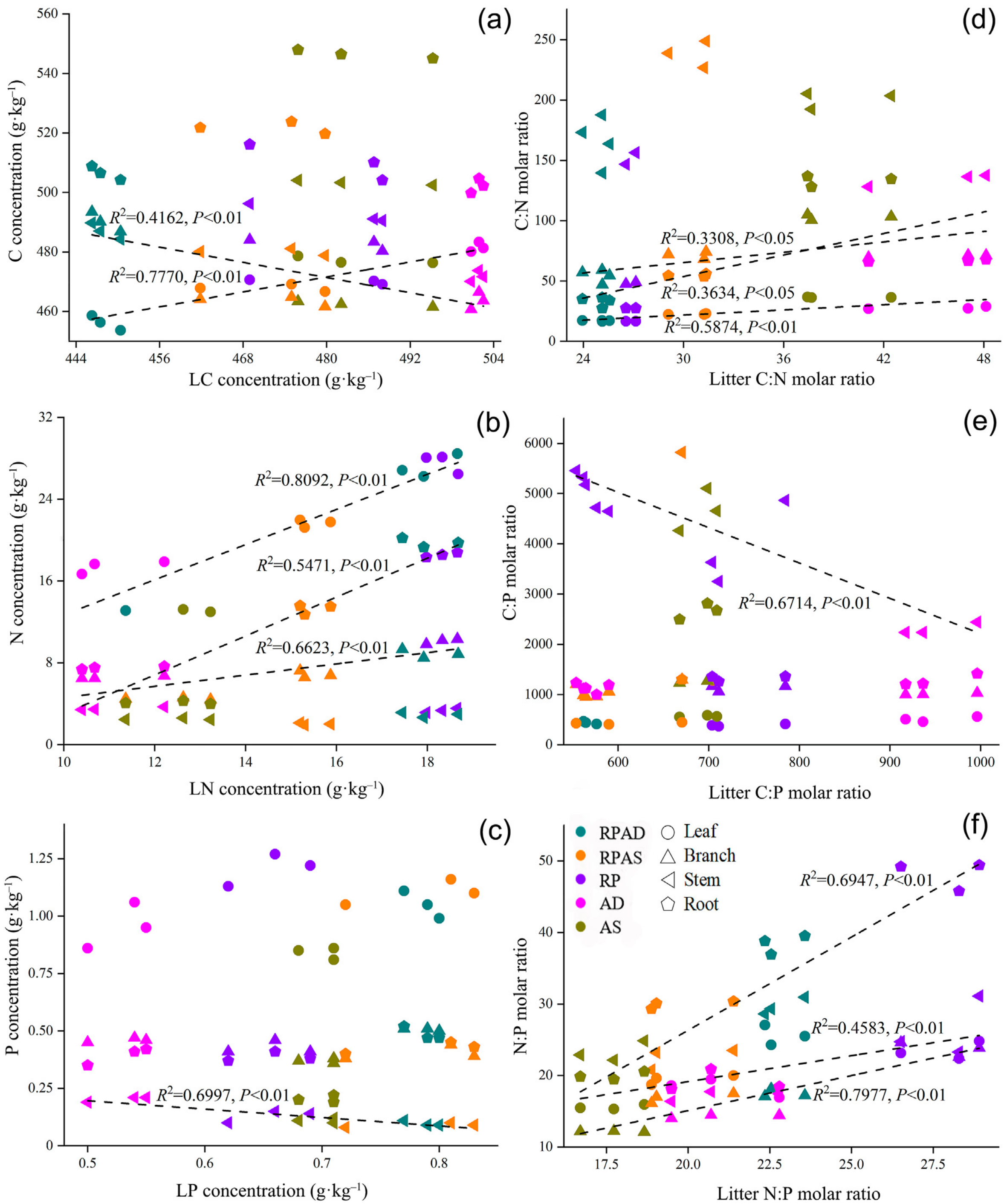

Fig. 6. Relationships between plant (leaf, branch, stem, fine root) and litter in C, N, and P concentrations (**a**, **b**, **c**) and stoichiometric ratio (**d**, **e**, **f**) of different plantations.

distributions of fine root systems in mixed plantations provide an enabling environment for rhizobium activities (Singh et al., 2012). Higher nitrogen availability frequently translates in higher plant-microbes capacity to mobilizate and uptake other nutrients such as phosphorus (Sardans and Peñuelas, 2012). Thus, mixing *R. pseudoacacia* with *A. sibirica* can improve N-cycling and the availability of soil phosphorus (Forrester et al., 2006).

The soil C:N and C:P ratios can indicate the soil nitrogen and phosphorus mineralization capacity, and a lower ratio demonstrates higher net nitrogen and phosphorus mineralization (Bui and Henderson, 2013; Marty et al., 2017). Compared with RPAD, RPAS had the higher net nitrogen and phosphorus mineralization (Fig. 3b and c), which is beneficial to alleviate plants growth limited by soil nitrogen and phosphorus. Zhou et al. (2019) reported that soil C:P ratio gradually decreased with deepening soil, consistent with our results. This is because SOC concentration was declined gradually in different plantations, while soil TP concentration was relatively stable across the soil profile (Fig. 3a, c) (Qiu et al., 2010; Yang et al., 2018; Pang et al., 2021). N:P ratio can reflect the status of soil nitrogen saturation and determine the threshold of nutrient limitation (Tessier and Raynal, 2003; Cui et al., 2021). Here, our data suggest that mixtures of *R. pseudoacacia* with *A. sibirica* could alleviate N-limitation issues (Fig. 3f) (Zhu et al., 2013).

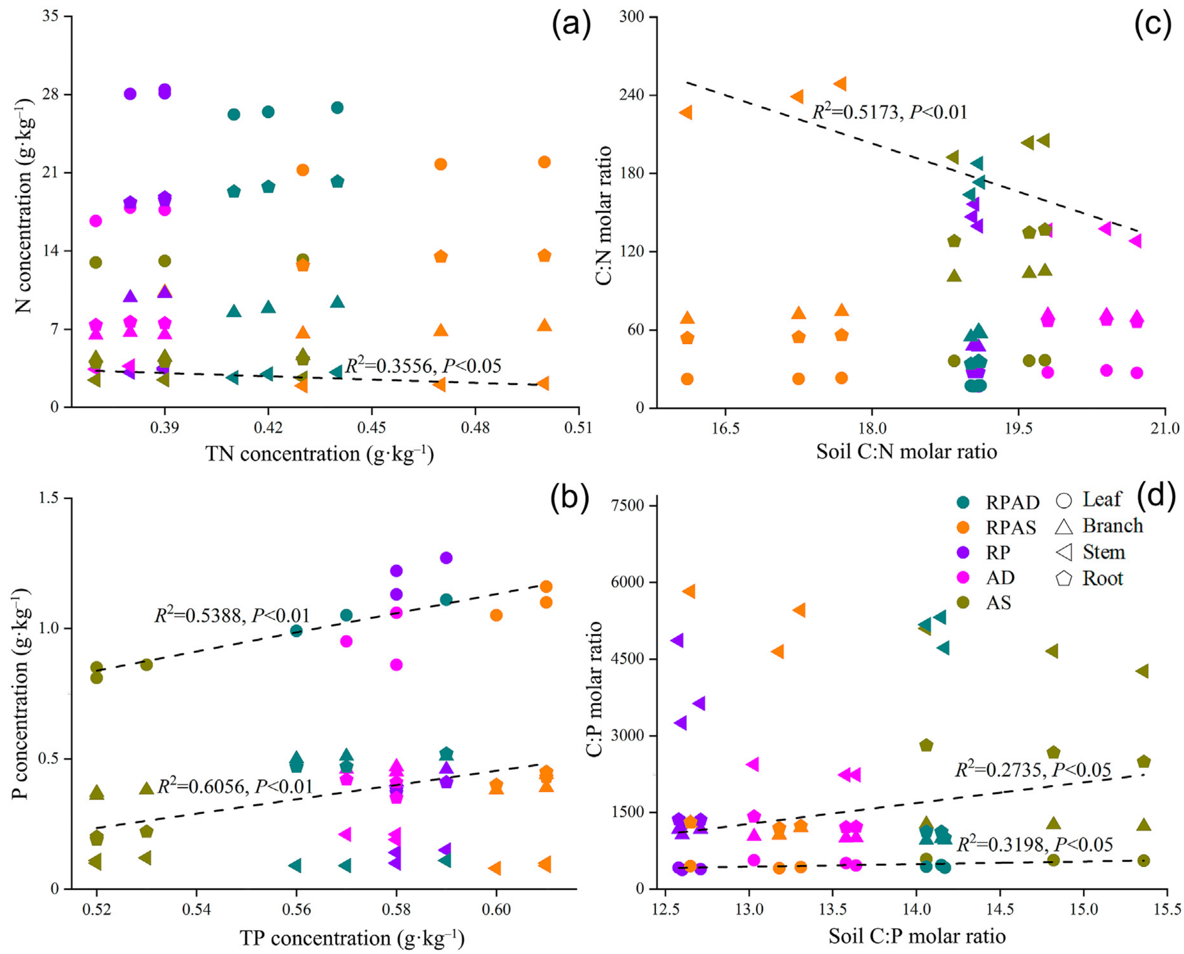

Fig. 7. Relationships between plant (leaf, branch, stem, fine root) and average soil in N and P concentrations (**a**, **b**) and stoichiometric ratio (**c**, **d**) of different plantations. No significant relationship was found between plant (leaf, branch, stem, fine root) and average soil in C concentrations and N:P ratio of different plantations.

Table 5
Litter and soil C:N:P stoichiometry compared to plant (leaf, branch, stem, fine root) C:N:P stoichiometry.

Fraction (%)	Leaf C:N:P stoichiometry		Branch C:N:P stoichiometry		Stem C:N:P stoichiometry		Root C:N:P stoichiometry	
	Concentrations	Ratios	Concentrations	Ratios	Concentrations	Ratios	Concentrations	Ratios
A	53.9	49.5	65.3	34.5	36.2	35.3	37.5	50.0
B	19.0	10.1	14.6	15.1	47.4	9.5	46.6	18.8
C	20.0	19.0	5.1	16.8	16.5	29.8	8.0	20.6

Notes: A and B represents the separate explained of litter and soil, respectively; C represent the combined explained of litter and soil.

4.2. Relationships between C:N:P stoichiometry in plant, litter, and soil of the mixed-species and monoculture plantations

Consistent with hypothesis 2, positive correlations were observed between N and P concentrations and between C:N and C:P ratios in plant structures (i.e., leaf, branch, stem, fine root) (Fig. 4c and d), indicating that plants were able to use the increased nitrogen to allocate more resources to phosphorus extraction from organic matter and promote soil P-cycling (Reich et al., 1992). Additionally, stem C:P ratio was positively correlated with the soil N:P ratio in RPAS, whereas similar results were not found in monocultures (Fig. 4f). This indicated that mixed-species afforestation regulated the effects of soil TN and TP on stem phosphorus use efficiency (Yin et al., 2021), which was consistent with previous studies (Yang et al., 2018; Wu et al., 2021b; Jiao et al., 2022).

In addition to a positive correlation between litter decomposition rates and initial nitrogen and phosphorus concentrations (Wang et al., 2008), annual litter production was higher in mixed plantations than in monocultures (Farooq et al., 2022). Therefore, both N- and P-cycling rates were faster in mixed-species plantations than in their respective monocultures, and the mixed-species plantations had more active biogeochemical cycles (Forrester et al., 2006). Furthermore, litter was positively correlated with the soil TN concentrations in mixed-species plantations ($P < 0.05$, Fig. 5a and b), while similar results were not found between litter and soil TP concentrations ($P > 0.05$, Fig. 5a and b). This is inconsistent with previous studies in which 90 % of the nitrogen and phosphorus elements released from litter into the soil were taken up by plants (Chapin et al., 2011). Wang et al. (2008) and Farooq et al. (2022) explained that nitrogen returned to the soil by litter accounted for 80 % of the total nutrients returning to the soil, while only 2 % of the total nutrients returned through the litter was phosphorus.

4.3. Effect of litter and soil on plant C:N:P stoichiometry among mixed-species and monoculture plantations

Previous studies have demonstrated that leaf-to-litter conversion occurs rapidly, and branches contain increased carbon reserves (Pang et al., 2021), which aligns with our finds (Fig. 6a, Table 5). Additionally, the observed relationships among plant organs (leaf, branch, and root) and litter N concentrations (Fig. 6b) and C:N ratios (Fig. 6d) strongly suggest that plants with high nitrogen requirements promote the rapid decomposition of litter (Bradford et al., 2016). This implies a coupling strategy of "nutrient demand-supply" (Guo et al., 2021). However, we only found negative correlations between litter and stem P concentration ($P < 0.01$, Fig. 6c) and C:P ratio ($P < 0.01$, Fig. 6e). Thus, after phosphorus is transported from the stem to the leaf, its transfer to other organs becomes challenging. N:P ratios demonstrated a stronger relationship between litter and roots compared to leaves and branches ($P < 0.01$, Fig. 6f; Table 5), further suggesting that most litter decomposition products are absorbed by roots, while only a portion of them is subsequently distributed to branches and leaves, consistent with previous studies (Chapin et al., 2011).

Consistent with hypothesis 3, we only observed negative correlations of N concentration and C:N ratio between stems and soil ($P < 0.05$, Fig. 7a–c), indicating that the stem is the plant organ more directly linked to soil TN concentrations (Table 5). However, carbon and nitrogen in leaf, branch, and root can also originate from sources other than direct uptake from the soil, such as photosynthetic carbon fixation, and biological nitrogen fixation (Méndez and Karlsson, 2005). Both leaves and roots are highly active plant organs, and their physiological traits are strongly associated with soil phosphorus (Ren et al., 2016), which is consistent with our findings (Fig. 7b). This suggests that soil TP concentrations play a crucial role in nutrient reallocation within leaves and roots over long-term processes. Therefore, all the results indicate that the majority of phosphorus released into the soil from litter is absorbed by the roots and subsequently transferred to the leaves.

5. Conclusions

In this study, we investigated the effects of afforestation patterns on ecological stoichiometry in the plant-litter-soil system, in the Loess Hilly Region of China. Our results reveal that trees maintain their biogeochemical niche when growing in mixed stands linked to more efficient nutrient use, e.g. alleviated soil nitrogen deficiency. Alleviated N-limitation of litter decomposition was influenced by individual tree species and afforestation patterns. In addition, soil TN and TP concentrations differed depending on the soil layer. In the tree organs (leaf, branch, stem, fine root) of different plantations, a positive correlation was shown between N and P concentrations, and thus between C:N and C:P ratios. Positive correlations between tree organs (i.e., leaf, branch, root) and litter for N concentration and C:N ratio, however, were reversed between stem and soil. Most of the nitrogen and phosphorus released into the soil from litter were absorbed by trees. Meanwhile, leaf and root obtained phosphorus mainly from soil, and nitrogen both from soil and biological N_2-fixation. These results are important for understanding the correlation of C:N:P stoichiometry between the plant-litter-soil system of different afforestation plantations and, in particular, for identifying mixed plantations, which provides insights into suitable tree species selection and plantation management.

Availability of data and materials

Data and materials can be obtained by contacting the corresponding author.

Funding

This study was funded by the National Nature Science Foundation of China (No. 41771556). JP and JS were funded by the Spanish Government Grants PID2020-115770RB-I00 and TED2021-132627B–I00 funded by the Spanish MCIN, AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR, the Catalan government grant SGR2021-1333 and the Fundación Ramón Areces grant CIVP20A6621.

Authors' contributions

YMC and SBL conceived and designed the study; SBL conducted the experiment; SBL, YMC, JS, and JP analyzed the results, wrote and edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fecs.2023.100123.

References

Bao, S.D., 2000. Soil and Agriculture Chemistry Analysis, third ed. China Agriculture Press, Beijing (in Chinese with English abstract).

Bauhus, J., Winden, A.P., Adrienne, B., Nicotra, A.B., 2004. Aboveground interactions and productivity in mixed-species plantations of *Acacia mearnsii* and *Eucalyptus globulus*. Can. J. For. Res. 34 (3), 686–694. https://doi.org/10.1139/x03-243.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen - total. In: Page, A.L. (Ed.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, Agronomy Monograph, vol. 9. ASA and SSSA, Madison.

Bradford, M.A., Berg, B., Maynard, D.S., Wieder, W.R., Wood, S.A., 2016. Understanding the dominant controls on litter decomposition. J. Ecol. 104, 229–238. https://doi.org/10.1111/1365-2745.12507.

Britton, A.J., Mitchell, R.J., Fisher, J.M., Riach, D.J., Taylor, A.F., 2018. Nitrogen deposition drives loss of moss cover in alpine moss-sedge heath via lowered C:N ratio and accelerated decomposition. New Phytol. 218, 470–478. https://doi.org/10.1111/nph.15006.

Bui, E.N., Henderson, B.L., 2013. C:N:P stoichiometry in Australian soils with respect to vegetation and environmental factors. Plant Soil 373 (1–2), 553–568. https://doi.org/10.1007/s11104-013-1823-9.

Camp, R.F., 1986. Walnuts and white pine can be growth together successfully. Tree Planters' Notes (U.S. Dep. Agric. For. Serv.) 37 (2), 29–31.

Cao, Y., Chen, Y.M., 2017. Coupling of plant and soil C:N:P stoichiometry in black locust (*Robinia pseudoacacia*) plantations on the Loess Plateau, China. Trees Struct. Funct. 31, 1559–1570. https://doi.org/10.1007/s00468-017-1569-8.

Chapin, F.S., Matson, P.A., Mooney, H.A., 2011. Principles of Terrestrial Ecosystem Ecology. Springer, New York.

Chapman, S.K., Newman, G.S., 2010. Biodiversity at the plant-soil interface: Microbial abundance and community structure respond to litter mixing. Oecologia. 162, 763–769. https://doi.org/10.1007/s00442-009-1498-3.

Coll, L., Ameztegui, A., Collet, C., Lof, M., Mason, B., Pach, M., Verheyen, K., Abrudan, I., Barbati, A., Barreiro, S., Bielak, K., Bravo-Oviedo, A., Ferrari, B., Govedaro, Z., Kulhavy, J., Lazdina, D., Metslaid, M., Mohren, F., Pereira, M., Peric, S., Rasztovits, E., Short, I., Spathelf, P., Sterba, H., Stojanovic, D., Valsta, L., Zlatanov, T., Ponette, Q., 2018. Knowledge gaps about mixed forests: what do European forest managers want to know and what answers can science provide? For. Ecol. Manag. 407, 106–115. https://doi.org/10.1016/j.foreco.2017.10.055.

Cui, H., Ou, Y., Wang, L.X., Liang, A.Z., Yan, B.X., Li, Y.X., 2021. Dynamic changes in microbial communities and nutrient stoichiometry associated with soil aggregate structure in restored wetlands. Catena 197, 104984. https://doi.org/10.1016/j.catena.2020.104984.

Dawud, S.M., Raulund-Rasmussen, K., Domisch, T., Finér, L., Jaroszewicz, B., Vesterdal, L., 2016. Is tree species diversity or species identity the more important driver of soil carbon stocks, C/N ratio, and pH? Ecosystems 19, 645–660. https://doi.org/10.1007/s10021-016-9958-1.

Eviner, V.T., Chapin, F.S., 2002. The influence of plant species, fertilization and elevated CO_2 on soil aggregate stability. Plant Soil 246, 211–219. https://doi.org/10.1023/A:1020657107687.

FAO, 2020. Global Forest Resources Assessment. Food and Agriculture Organization of the United Nations.

Farooq, T.H., Li, Z., Yan, W., Shakoor, A., Kumar, U., Shabbir, R., Peng, Y., Gayathiri, E., Alotaibi, S.S., Wróbel, J., Kalaji, H.M., Chen, X., 2022. Variations in litterfall dynamics, C:N:P stoichiometry and associated nutrient return in pure and mixed stands of camphor tree and Masson pine forests. Front. Environ. Sci. 10, 903039. https://doi.org/10.3389/fenvs.2022.903039.

Feng, X.M., Fu, B.J., Piao, S.L., Wang, S., Ciais, P., Zeng, Z.Z., Lü, Y.H., Zeng, Y., Li, Y., Jiang, X.H., Wu, B.F., 2016. Revegetation in China's Loess Plateau is approaching sustainable water resource limits. Nat. Clim. Change 6, 1019–1022. https://doi.org/10.1038/nclimate3092.

Forrester, D.I., 2014. The spatial and temporal dynamics of species interactions in mixed-species forests: from pattern to process. For. Ecol. Manag. 312, 282–292. https://doi.org/10.1016/j.foreco.2013.10.003.

Forrester, D.I., Bauhus, J., Cowie, A.L., 2006. On the success and failure of mixed-species tree plantations: lessons learned from a model system of *Eucalyptus globulus* and *Acacia mearnsii*. For. Ecol. Manag. 209 (1–2), 147–155. https://doi.org/10.1016/j.foreco.2005.01.012.

Fu, B.J., Wang, S., Liu, Y., Liu, J., Liang, W., Miao, C., 2017. Hydrogeomorphic ecosystem responses to natural and anthropogenic changes in the Loess Plateau of China. Annu. Rev. Earth Planet Sci. 45 (1), 223–243. https://doi.org/10.1146/annurev-earth-063016-020552.

Gargallo-Garriga, A., Sardans, J., Pérez-Trujillo, M., Oravec, M., Urban, O., Jebtsch, A., Kreyling, J., Beierkuhnlein, C., Parella, T., Peñuelas, J., 2015. Warming differentially influences the effects of drought on stoichiometry and metabolomics in shoots and roots. New Phytol. 207, 59–1603. https://doi.org/10.1111/nph.13377.

Gargallo-Garriga, A., Sardans, J., Pérez-Trujillo, M., Rivas-Ubach, A., Oravec, M., Veceerova, K., Urban, O., Jentsch, A., Kreyling, J., Beierkuhnlein, C., Parella, T., Peñuelas, J., 2014. Opposite metabolic responses of shoots and roots to drought. Sci. Rep. 4, 6829. https://doi.org/10.1038/srep06829.

Guo, L.L., Deng, M.F., Yang, S., Liu, W.X., Wang, X., Wang, J., Liu, L.L., 2021. The coordination between leaf and fine root litter decomposition and the difference in their controlling factors. Global Ecol. Biogeogr. 30, 2286–2296. https://doi.org/10.1111/geb.13384.

Güsewell, S., 2004. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164 (2), 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x.

Gusewell, S., Freeman, C., 2005. Nutrient limitation and enzyme activities during litter decomposition of nine wetland species in relation to litter N:P ratios. Funct. Ecol. 19 (4), 582–593. https://doi.org/10.1111/j.1365-2435.2005.01002.x.

Güsewell, S., Verhoeven, J.T.A., 2006. Litter N:P ratios indicate whether N or P limits the decomposability of graminoid leaf litter. Plant Soil 287 (1–2), 131–143. https://doi.org/10.1007/s11104-006-9050-2.

Han, W.X., Tang, L.Y., Chen, Y.H., Fang, J.Y., 2013. Relationship between the relative limitation and resorption efficiency of nitrogen vs phosphorus in woody plants. PLoS One 8 (12), e83366. https://doi.org/10.1371/journal.pone.0083366.

He, M.Z., Dijkstra, F.A., 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. New Phytol. 204, 924–931. https://doi.org/10.1111/nph.12952.

Hou, L., Dong, Z.J., Yang, Y.Y., Zhang, D.H., Zhang, S.L., Zhang, S.X., 2018. Applying foliar stoichiometric traits of plants to determine fertilization for a mixed pine-oak stand in the Qinling Mountains, China. PeerJ 6, e4628. https://doi.org/10.7717/peerj.4628.

Jiao, Q.Y., Huang, L.J., Zhang, J.J., Zhang, X., Huang, L., Guo, Z., Cao, Y., 2022. Ecological stoichiometry and carbon storage of *Robinia pseudoacacia* mixed forests in Hilly-gully area of the Loess Plateau. J. Soil Water Conserv. 36 (2), 238–246. https://doi.org/10.13870/j.cnki.stbcxb.2022.02.030 (in Chinese with English abstract).

Khanna, M., Dhungana, B., Clifton-Brown, J., 2008. Costs of producing miscanthus and switchgrass for bioenergy in Illinois. Biomass Bioenergy 32, 482–493. https://doi.org/10.1016/j.biombioe.2007.11.003.

Laclau, J., Levillain, J., Deleporte, P., Nzila, J.D., Bouillet, J., Saint-André, L., Versini, A., Mareschal, L., Nouvellon, Y., M'Bou, A.T., Ranger, J., 2010. Organic residue mass at planting is an excellent predictor of tree growth in *Eucalyptus* plantations established on a sandy tropical soil. For. Ecol. Manag. 260, 2148–2159. https://doi.org/10.1016/j.foreco.2010.09.007.

Liu, G.B., Shangguan, Z.P., Yao, W.Y., Yang, Q.K., Zhao, M.J., Dang, X.H., Guo, M.H., Wang, G.L., Wang, B., 2017. Ecological effects of soil conservation in Loess Plateau. Bull. Chin. Acad. Sci. 32, 11–19. https://doi.org/10.1080/13504500609469682.

Lü, X.T., Freschet, G.T., Flynn, D.F., Han, X.G., 2012. Plasticity in leaf and stem nutrient resorption proficiency potentially reinforces plant-soil feedbacks and microscale heterogeneity in a semi-arid grassland. J. Ecol. 100, 144–150. https://doi.org/10.1111/j.1365-2745.2011.01881.x.

Liu, L., Ge, J.L., Shu, H.W., Zhao, C.M., Xu, W.T., Shen, G.Z., Xie, Z.Q., 2019. C, N and P stoichiometric ratios in mixed evergreen and decciduous broad leaved forests in Shennongjia, China. J. Plant Ecol. 43, 482–489. https://doi.org/10.17521/cjpe.2019.0064.

Manzoni, S., Trofymow, J.A., Jackson, R.B., Porporato, A., 2010. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecol. Monogr. 80 (1), 89–106. https://doi.org/10.1890/09-0179.1.

Marty, C., Houle, D., Gagnon, C., Courchesne, F., 2017. The relationships of soil total nitrogen concentrations, pools and C:N ratios with climate, vegetation types and nitrate deposition in temperate and boreal forests of eastern Canada. Catena 152, 163–172. https://doi.org/10.1016/j.catena.2017.01.014.

Méndez, M., Karlsson, P.S., 2005. Nutrient stoichiometry in *Pinguicula vulgaris*: nutrient availability, plant size, and reproductive status. Ecology 86 (4), 982–991. https://doi.org/10.1890/04-0354.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Nelson, D., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, Agronomy Monograph, vol. 9. ASA and SSSA, Madison.

Pang, Y., Tian, J., Liu, L.X., Han, L.N., Wang, D.X., 2021. Coupling of different plant functional group, soil, and litter nutrients in a natural secondary mixed forest in the Qinling Mountains, China. Environ. Sci. Pollut. Res. 28, 66272–66286. https://doi.org/10.1007/s11356-021-15632-5.

Peñuelas, J., Baldocchi, D., 2019. Life and the five biological laws. Lessons for global change models and sustainability. Ecol. Complex. 38, 11–14. https://doi.org/10.1016/j.ecocom.2019.02.001.

Qiu, L.P., Zhang, X.C., Cheng, J.M., Yin, X.Q., 2010. Effects of black locust (*Robinia pseudoacacia*) on soil properties in the loessial gully region of the Loess Plateau, China. Plant Soil 332, 207–217. https://doi.org/10.1007/s11104-010-0286-5.

Reich, P.B., Walters, M.B., Ellsworth, D.S., 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecol. Monogr. 62 (3), 365–392. https://doi.org/10.2307/2937116.

Ren, C.J., Zhao, F.Z., Kang, D., Yang, G.H., Han, X.H., Tong, X.G., Feng, Y.Z., Ren, G.X., 2016. Linkages of C:N:P stoichiometry and bacterial community in soil following afforestation of former farmland. For. Ecol. Manag. 376, 59–66. https://doi.org/10.1016/j.foreco.2016.06.004.

Richard, C., Engelbrecht, B.M.J., Delicia, P., Rolando, P., Turner, B.L., 2013. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. Proc. Natl. Acad. Sci. U.S.A. 110, 5064–5068. https://doi.org/10.1073/pnas.1218042110.

Sardans, J., Grau, O., Chen, H.Y.H., Janssens, I.A., Ciais, P., Piao, S.L., Peñuelas, J., 2017. Changes in nutrient concentrations of leaves and roots in response to global change factors. Global Change Biol. 23, 3849–3856. https://doi.org/10.1111/gcb.13721.

Sardans, J., Peñuelas, J., 2012. The role of plants in the effects of global change on nutrient availability and stoichiometry. Plant Physiol. 160, 1741–1761. https://doi.org/10.1104/pp.112.208785.

Sariyildiz, T., Anderson, J.M., 2003. Interactions between litter quality, decomposition and soil fertility: a laboratory study Soil. Biol. Biochem. 35, 391–399. https://doi.org/10.1016/S0038-0717(02)00290-0.

Shan, C., Liang, Z., Hao, W., 2002. Review on growth of locust and soil water in Loess Plateau. Acta Bot. Boreali Occident. Sin. 23, 1341–1346 (in Chinese with English abstract).

Singh, K., Singh, B., Singh, R.R., 2012. Changes in physico-chemical, microbial and enzymatic activities during restoration of degraded sodic land: ecological suitability of mixed forest over monoculture plantation. Catena 96, 57–67. https://doi.org/10.1016/j.catena.2012.04.007.

Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton.

Tessier, A.J.T., Raynal, D.J., 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J. Appl. Ecol. 40 (3), 523–534. https://doi.org/10.1046/j.1365-2664.2003.00820.x.

ter Braak, T., Smilauer, P., 2012. Canoco Reference Manual and User's Guide: Software for Ordination, Version 5.0. Microcomputer Power, Ithaca.

Townsend, A.R., Cleveland, C.C., Asner, G.P., Bustamante, M.M.C., 2007. Controls over foliar N:P ratios in tropical rain forests. Ecology 88, 107–118. https://doi.org/10.1890/0012-9658(2007)88[107:cofnri]2.0.co;2.

Wang, Q.K., Wang, S.L., Huang, Y., 2008. Comparisons of litterfall, litter decomposition and nutrient return in a monoculture *Cunninghamia Lanceolata* and a mixed stand in Southern China. For. Ecol. Manag. 255, 1210–1218. https://doi.org/10.1016/j.foreco.2007.10.026.

Wang, Z.N., Lu, J.Y., Zhang, Q.P., 2015. Stoichiometric characteristics of carbon, nitrogen, and phosphorus in leaves of differently aged lucerne (*Medicago sativa*) stands. Front. Plant Sci. 6, 1062. https://doi.org/10.3389/fpls.2015.01062.

Wei, J., Li, Z., Feng, X., Zhang, Y., Chen, W., Wu, X., Jiao, L., Wamg, X., 2018. Ecological and physiological mechanisms of growth decline of *Robinia pseudoacacia* plantations in the Loess Plateau of China: a review. Chin. J. Appl. Ecol. 29, 2433–2444 (in Chinese with English abstract).

Wojciech, P., Ewa, B., Martin, L., 2019. A comparison of C:N:P stoichiometry in soil and deadwood at an advanced decomposition stage. Catena 179, 1–5. https://doi.org/10.1016/j.catena.2019.03.025.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., 2004. The worldwide leaf economics spectrum. Nature 428, 821–827. https://doi.org/10.1038/nature02403.

Wu, X., Niu, Y.B., Xun, M., Jin, J., Tang, Y.K., Chen, Y.M., 2021a. Soil carbon, nitrogen, and phosphorus storages and their stoichiometry due to mixed afforestation with *Hippophae rhamnoides* in the Loess Hilly Region, China. Forests 12 (12), 1718. https://doi.org/10.3390/f12121718.

Wu, X., Niu, Y.B., Chen, Y.-M., Dong, C.G., Qiao, Y.L., 2021b. Carbon, nitrogen and phosphorus stoichiometry characteristics in leaf, litter and soil at mixed forests of *Hippophae rhamnoides* in the Loess Hilly Region of China. J. Soil Water Conserv. 35 (4), 369–376. https://doi.org/10.13870/j.cnki.stbcxb.2021.04.050 (in Chinese with English abstract).

Yang, Y., Dou, Y.X., An, S.S., 2018. Testing association between soil bacterial diversity and soil carbon storage on the Loess Plateau. Sci. Total Environ. 626, 48–58. https://doi.org/10.1016/j.scitotenv.2018.01.081.

Yin, H., Zheng, H.W., Zhang, B., Zhang, B., Tariq, A., Lv, G.H., Zeng, F.J., Graciano, C., 2021. Stoichiometry of C:N:P in the roots of *Alhagi sparsifolia* is more sensitive to soil nutrients than aboveground organs. Front. Plant Sci. 12, 698961. https://doi.org/10.3389/fpls.2021.698961.

Yuan, Z.Y., Chen, H.Y.H., Reich, P.B., 2011. Global-scale latitudinal patterns of plant fine-root nitrogen and phosphorus. Nat. Commun. 2, 344. https://doi.org/10.1038/ncomms1346.

Zeng, W.S., Chen, X.Y., Pu, Y., Yang, Y., 2018. Comparison of different methods for estimating forest biomass and carbon storage based on national forest inventory data. For. Res. 31 (1), 66–71. https://doi.org/10.13275/j.cnki.lykxyj.2018.01.008.

Zhang, D.J., Li, J.J., Huang, Y.M., Gao, S., Zhang, J., 2022. Root-soil facilitation in mixed *Eucalyptus grandis* plantations including nitrogen-fixing species. For. Ecol. Manag. 516, 120215. https://doi.org/10.1016/j.foreco.2022.120215.

Zhang, W., Liu, W.C., Xu, M.-P., Deng, J., Han, X.H., Yang, G.H., Feng, Y.Z., Ren, G.X., 2019. Response of forest growth to C:N:P stoichiometry in plants and soils during *Robinia pseudoacacia* afforestation on the Loess Plateau, China. Geoderma 337, 280–289. https://doi.org/10.1016/j.geoderma.2018.09.042 (in Chinese with English abstract).

Zhao, B.S., Li, S.X., Li, S.Q., 1995. The causes of the differences of legume and non-legume crop responses to P fertilizers. Agric. Res. Arid Areas 13 (2), 45–56 (in Chinese with English abstract).

Zhou, Z.H., Wang, C.K., Jin, Y., Sun, Z.H., 2019. Impacts of thinning on soil carbon and nutrients and related extracellular enzymes in a larch plantation. For. Ecol. Manag. 450, 117523. https://doi.org/10.1016/j.foreco.2019.117523.

Zhu, Q.L., Xing, X.Y., Zhang, H., An, S.S., 2013. Soil ecological stoichiometry under different vegetation area on loess hilly-gully region. Acta Ecol. Sin. 33, 4674–4682. https://doi.org/10.5846/stxb201212101772 (in Chinese with English abstract).