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Response of water use efficiency and plant-soil C:N:P stoichiometry to stand quality in *Robinia pseudoacacia* on the Loess Plateau of China

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ABSTRACT

The evaluation of stand quality is of great significance for forest management and sustainable development. Water use efficiency (WUE) and the carbon (C), nitrogen (N), and phosphorus (P) stoichiometric characteristics of plants and soil are important indexes used to evaluate plant water and nutrients adaptation strategies. However, little is known about the effects of stand quality on WUE and plant soil C:N:P stoichiometry. In this study, the growth characteristics of *Robinia pseudoacacia* plantations and understory plants in an age series of 8-, 15-, 25-, and 35-year-old stands were investigated, and the leaf-level WUE and C:N:P stoichiometry in plants and soil were measured on the Loess Plateau. The results showed that the species richness, Shannon-Weiner diversity index, and evenness index reached their peaks in the 35-year-old stands with increasing afforestation age. WUE reached its highest value in the 35-year-old stands but was only significantly higher than that in the 8-year-old stands. Stand age had different effects on C, N, and P nutrients and stoichiometry in leaves, fine roots, and soil. The most obvious trend was that soil total P decreased significantly with increasing afforestation age, and the leaf N:P ratio was greater than 16 in the four stand ages, which indicated that P was the main limiting factor in *R. pseudoacacia* plantations. Leaf nutrients and stoichiometry are closely related to forest growth characteristics, while root nutrients and stoichiometry are more related to understory plant composition and diversity. Importantly, the WUE had no significant change with the increase in the stand quality index, and the response of soil stoichiometry to stand quality was stronger than that of plant stoichiometry. These results help us further understand coordinated plant-soil restoration after afforestation.

1. Introduction

Afforestation is a common and effective method used to curb soil degradation, improve the ecological environment and restore degraded ecosystems, and it plays a crucial role in regulating climate change (Deng et al., 2017; Yu et al., 2020). This practice of human land-use change has greatly affected the aboveground vegetation and underground soil ecosystem (Ren et al., 2017; Zhang et al., 2019a) and has changed the circulation of water and mineral nutrients between the aboveground and underground ecosystems (Bai et al., 2019; Jiang et al., 2019; Zhong et al., 2020). Ecostoichiometry, which focuses on the balance and interaction of chemical elements in ecological processes (Elser et al., 2000; Wardle et al., 2004a), is often used to study the feedback between aboveground and underground ecological components and the coupling relationship between elements (Delgado-Baquerizo et al., 2017; Zechmeister-Boltenstern et al., 2015). Several studies have used the stoichiometric characteristics of soil and plant tissues at regional or global scales to indicate soil fertility and plant nutrition status (Maaroufi and De Long, 2020; Xu et al., 2013; Yuan et al., 2011; Zhang et al., 2018), thus revealing the nutrient cycle between plants and soil; additionally, they have used the C, N, and P stoichiometric relationship between plants and soil as the main ecological index to understand ecosystem functions and processes (Cao and Chen, 2017; Elser, 2006).

Water use efficiency (WUE), which defines the water adaptation strategies of plants, is not only an important indicator to evaluate the adaptability of plants to environmental changes but also a key factor to reflect the trade-off between carbon assimilation and water dissipation (Beer et al., 2009; Gao et al., 2014). In recent years, increasing attention has been given to WUE at the leaf scale because the study of WUE at the leaf level can reveal the internal water consumption mechanism of

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plants. Leaf carbon isotopes are often used as an indicator to estimate long-term plant WUE (Frank et al., 2015; Franks et al., 2013). Previous studies have shown that WUE changes greatly in time and space due to changes in climate, soil, vegetation types, and topography (Hatfield and Dold, 2019; Niu et al., 2011; Wang et al., 2020). Therefore, research on how plant WUE changes with vegetation succession will provide further insights into the water use strategy in the process of afforestation and the sustainable development of afforestation.

Afforestation can affect the stand structure, primary productivity, and resource use efficiency of plant communities, leading to changes in ecosystem functions and soil quality (Esteban Lucas-Borja and Delgado-Baquerizo, 2019; Lazzaro et al., 2018; Rosenfield and Muller, 2020; Sanji et al., 2020). However, these studies usually focus on dominant tree species and ignore the contribution of understory plants. As an important part of forest ecosystems (Nilsson and Wardle, 2005), understory vegetation not only plays a unique role in maintaining the stability of forest ecological function and biodiversity (Gilliam, 2007) but also affects the succession dynamics and ecosystem characteristics of forest communities by competing with forests for the availability of water and nutrient resources (Barbier et al., 2008; Majasalmi and Rautiainen, 2020). For example, forest growth has a positive response to nitrogen fertilizer, and with the increase in nitrogen, the nitrogen limitation of forests has been alleviated (Hedwall et al., 2018; Tian et al., 2018), but the growth of understory plants in these forest ecosystems has a negative response to nitrogen input, such as a decrease in understory biomass and a change in community structure (Dirnboeck et al., 2014; Xing et al., 2019). Thus, it is very important to clarify the relationship between the growth characteristics of trees and understory plants and the water and nutrient use of trees to understand the changes in the ecosystem material cycle during the development of plantations.

Due to its unique geographical location, climate, topography, and human activities, the Loess Plateau has become one of the most serious soil erosion areas in China and even globally (Fu et al., 2017). To control soil erosion and restore the ecological environment, the Chinese government has implemented a series of measures since 1950. Vegetation restoration is considered to be one of the most effective strategies to restore disturbed ecosystems (Zheng, 2006). The Grain for Green program implemented in 1999 is the largest afforestation program in China (Feng et al., 2016). With the implementation of the program of the conversion of sloping farmland to forest, the vegetation coverage increased significantly, which effectively restrained the further deterioration of soil erosion (Chen et al., 2015; Zhou et al., 2012). Although great achievements have been made in the ecological construction of the Loess Plateau, the problems of forest degradation and unsustainable plantation development have become increasingly prominent, and the evaluation of forest quality has attracted attention (Yang et al., 2020; Zhao et al., 2019). Forest quality encompasses a wide range of contents and indicators, and there is currently no unified evaluation framework system. Due to the differences in the research area and research focus, the evaluation system established by different researchers is also different. Forest quality at the stand level refers to the internal structure and function of the forest (Li et al., 2020). The evaluation of forest ecosystem function is the main information used for stand quality evaluation, such as reducing the adverse effects of land use activities (Kumarasiri et al., 2021). Prior studies mainly focused on the effects of afforestation on vegetation characteristics, soil physicochemical properties, and microbiological characteristics to understand the ecological restoration process after afforestation (Liu et al., 2020a; Xu et al., 2020; Zhong et al., 2020), but corresponding research on forest quality remains inadequate. Studies of how reforestation affects forest quality have found that a decrease in vegetation cover and low rates of long-term tree survival due to improper tree selection and excessive tree density in Northern China (Cao et al., 2011). The proportion of good-quality and poor-quality *R. pseudoacacia* forests were evaluated in Yongshou County in Shaanxi Province on the Loess Plateau of China in a recent study (Zhao et al., 2019). Li et al. (2020) conducted an analysis combining forest quality data with socio-economic data to compare the difference in forest quality between village that did and did not implement reforestation program. These studies provide the theoretical basis for forest management and guidance for regional reforestation. However, few studies have examined the responses of plant WUE and plant-soil C:N:P stoichiometry to stand quality.

R. pseudoacacia, one of the most widely planted woody species in the world (Vitkova et al., 2017), has become one of the main pioneer species on the Loess Plateau because of its nitrogen fixation ability, rapid growth rate, and drought tolerance. In this study, we hypothesized that forest stand age would affect tree growth characteristics and understory species composition, thereby affecting plant water and nutrient use. Furthermore, we hypothesized that plant WUE and plant-soil C:N:P stoichiometry might respond to stand quality. Based on these hypotheses, we evaluated the effects of the growth of *R. pseudoacacia* plantations of different ages (8 years, 15 years, 25 years, and 35 years) on WUE and C:N:P stoichiometry. The objectives of this study were to assess (1) the effect of afforestation age on WUE and plant-soil C:N:P stoichiometry; (2) the relationship between the growth characteristics of forest and understory plants and WUE and plant-soil C:N:P stoichiometry; and (3) the response of plant WUE and plant-soil C:N:P stoichiometry to stand quality index. The results of this study may provide an important supplement for exploring the mechanism of plant environmental adaptation and provide new insights for evaluating the success of ecological restoration after afforestation.

2. Materials and methods

2.1. Study site

The field experiment was conducted in the Wangdonggou experimental watershed, Changwu County (34°59-35°18′ N, 107°38′-107°58′ E), Shaanxi Province, Northwest China, which is located in the south-central Loess Plateau. The climate in this area is a warm temperate semi-humid continental monsoon climate. The mean annual precipitation is 584 mm, and the annual variation in precipitation is large and uneven, mostly occurring from July to September. The mean annual temperature is 9.1 , and the mean annual frost-free period is 171 days. The soil is Cumuli-Ustic Isohumosols. As a key area for the construction of the Three North Shelterbelt Project and the comprehensive control and development of soil and water loss, Changwu has carried out large-scale afforestation activities. After decades of artificial vegetation restoration, the vegetation cover of *R. pseudoacacia* has increased significantly and become the dominant tree species in this area. In addition, alfalfa (Medicago sativa) is the main forage type, and apple (Malus pumila) is the main fruit resource. This area is part of the dry farming area, and the main planting system is wheat-wheat-maize rotation and continuous wheat.

2.2. Experimental design

The study was conducted from August to September 2018. According to the guidance of local forestry personnel and field investigations, *R. pseudoacacia* plantations with ages of 8 years, 15 years, 25 years and 35 years were randomly selected by using the method of space instead of time. The location of sampling points is shown in Fig. 1. Three plots of 20 m × 20 m were randomly established for the subsequent vegetation survey and vegetation and soil sampling. Detailed information about each age category of the *R. pseudoacacia* forest is given in Table 1.

2.3. Vegetation survey and sampling

Stand density and canopy density (CD) were investigated in each plot, and representative trees were selected to record their tree height and diameter at breast height (DBH). The mature leaves of *R. pseudoacacia* were collected from different directions in the middle

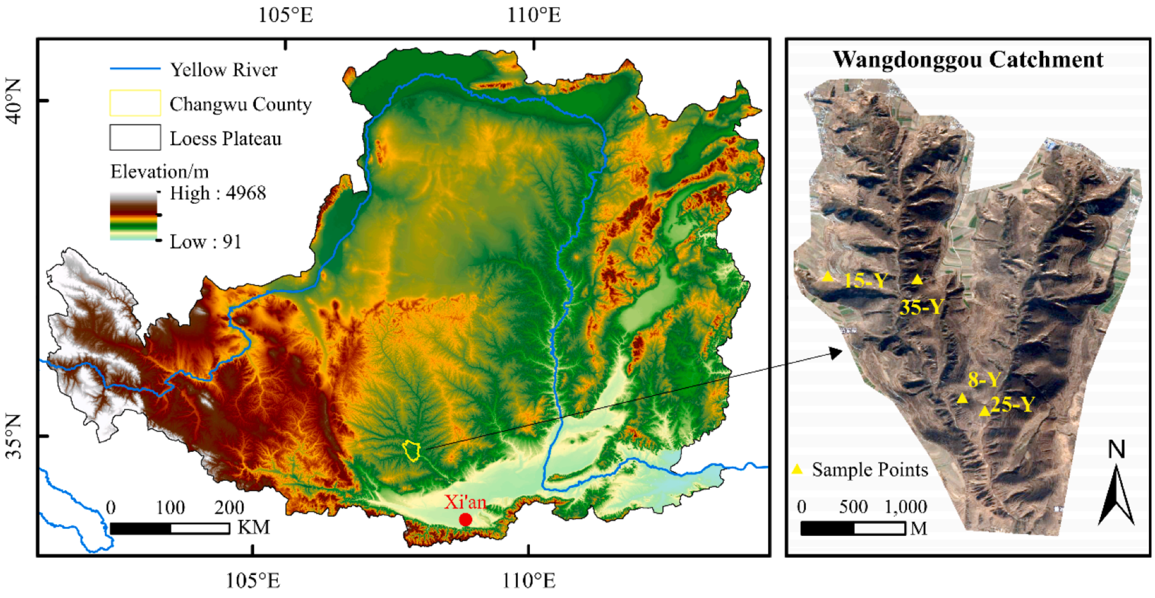

Fig. 1. Location of sample points on the Loess Plateau of China.

Table 1
Geographical information and plant communities of different stand age sites.

Site^{a}	Longitude	Latitude	Elevation/ m	Slope degree/°	Aspect	Stand density (number·hm^{-2})	Canopy density (%)	Tree height (m)	Diameter at breast height (cm)	Undergrowth dominant species
8-Y	107°41′36.804′′E	35°12′55.044′′N	1150	13.6	semi-shady	2000	75	9	5.17	*A. anomala, P. chinensis*
15-Y	107°40′54.200′′E	35°13′33.300′′N	1133	40.0	semi-sunny	2100	80	6	15.67	*A. anomala, A. sinicus*
25-Y	107°41′43.284′′E	35°12′51.702′′N	1122	35.7	semi-shady	2222	70	12	12.97	*A. sinicus, A. anomala*
35-Y	107°41′22.800′′E	35°13′32.472′′N	1158	43.3	shady	2500	90	15	16.50	*R. parvifolius, A. anomala*

Note: [a] indicates four *Robinia pseudoacacia* forests that were restored for 8 years, 15 years, 25 years and 35 years. *A. anomala*: *Arundinella anomala*, *P. chinensis*: *Potentilla chinensis*, *A. sinicus*: *Astragalus sinicus*, *R. parvifolius*: *Rubus parvifolius*.

and upper parts of the crown, and the leaves were evenly mixed and packed in kraft paper bags. After removing the litter on the soil surface, five soil samples (0–20 cm) were obtained with a stainless-steel auger (inner diameter of 5 cm) at a distance of 0.5 m from the base of the tree trunk, and each sample plot was evenly mixed to form a composite soil sample. These samples were sieved by 2 mm to remove stones, roots and debris. A portion of each soil sample was used to measure the soil water content (SWC). The remaining soil samples were dried for soil physical and chemical analysis. Soil bulk density (SBD) samples were randomly collected from three points of each plot by a 5-cm diameter cylinder core sampler. A root drill with a diameter of 9 cm was used to take root samples of 0–20 cm, and fine roots (<2 mm) were selected and stored in plastic bags. When selecting roots, attention should be given to distinguishing arbor roots and grass roots, and accurate judgment should be made by root color, root taste and characteristics. In addition, three 1 × 1 m squares were randomly arranged in each plot to estimate the species richness (R), Shannon-Wiener diversity index (H), Simpson diversity index (D) and Pielou evenness index (E). The calculation equations are as follows (Gotelli and Chao, 2013; Hao et al., 2016):

$$R = N \tag{1}$$

$$H = -\sum_{i=1}^{n}(n_i/N)\ln(n_i/N) \tag{2}$$

$$D = 1 - \sum_{i=1}^{n}(n_i/N)^2 \tag{3}$$

$$E = H/\ln R \tag{4}$$

where n_i is the number of individuals of species i and N is the total number of individuals of all species in the grassland community.

2.4. Determination of leaf, root and soil CNP and leaf carbon isotopes

The soil pH was determined in a soil/water (1:2.5; w/v) suspension with a pH meter. The SWC was determined by drying soil samples to constant mass in an oven at 105 . The SBD was determined by the soil core method, and the ratio of soil mass to total volume was calculated after drying to constant weight in an oven at 105 . Soil particle composition was measured by a Mastersizer-2000 (Malvern Instruments, Malvern, England). The soil organic carbon (SOC) content was determined by the dichromate oxidation method (Nelson and Sommers, 1982), the soil total nitrogen (TN) content was determined by the Kjeldahl method (Bremner and Mulvaney, 1982), and the soil total phosphorus (TP) and available phosphorus (AP) contents were determined by the molybdenum blue method (Murphy and Riley, 1962). Nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) were analyzed using a continuous flow analysis system (Autoanalyzer 3, Bran and Luebbe, Germany). The plant samples were dried at 105 for 30 min and then dried at 70 to constant weight. Then, the leaves and fine roots were ground into powder to analyze the contents of C, N, and P and the carbon isotopes of the leaves. The C concentration measurements were digested in a $K_2Cr_2O_7$-H_2SO_4 solution, heated with an oil bath, and then determined by titration. After digestion with H_2SO_4-H_2O_2, the N and P concentrations in plant samples were determined by the Kjeldahl method (Kjeltec 2300 Analyser Unit, Foss Tecator, Hoganas, Sweden) and the molybdenum yellow method (U-2800 spectrophotometer, China, Shanghai), respectively. Leaf carbon isotopes ($\delta^{13}C$) were analyzed using an isotope ratio mass spectrometer (Vario Pyro Cube, Elementar, Germany). The detailed calculations of the $\delta^{13}C$ and WUE values were performed according to our previous work (Su and Shangguan, 2020) .

2.5. Calculation of stand quality index

The stand quality index (SQI) is a comprehensive index reflecting the difference in stand quality among different stands, and its value ranges between 0 and 1. The process of stand quality evaluation was divided into four steps that include index selection, data quantification and standardization, index weight calculation and quality index calculation. Based on field survey data, the following indexes were selected to evaluate the stand quality of *R. pseudoacacia*: (1) investigation of forest structure, including average tree height, average DBH, stand density and CD; and (2) investigation of understory plants, including R, H and E. In addition, slope aspect was considered because slope aspect can significantly affect the water balance budget of forest, thus affecting forest growth (Wang et al., 2011). For the quantitative indicators, the original data were directly used; for the qualitative indicators, the equidistant assignment method was used, and slope aspect factors were assigned according to the duration of sunshine as follows: sunny slope with 1.0, semi-sunny slope with 2.0, semi-shady slope with 3.0 and shady slope with 4.0. It was necessary to standardize the data of each index due to the difference in the dimensions and positive and negative orientations of different evaluation indexes. In this study, the range method (0–1 standard method) was used for standardization, and the weight coefficient of each index was obtained by principal component analysis. The stand quality could be calculated by the following formula (Li et al., 2020):

$$SQI = \sum_{i=1}^{n} \left(D_i * W_{D_i}^{'}\right)\left(i \in [1, n]\right) \quad (5)$$

where D_i is the standard value of the i_{th} index, $W_{D_i}^{'}$ is the weight value of the i_{th} index, and n is the number of indexes.

2.6. Statistical analysis

One-way ANOVA was used to study the effects of stand age on grow characteristics of understory plants, soil physical and chemical properties, WUE and plant nutrients. Least squares difference (LSD) post hoc tests were conducted to identify significant differences between means. Pearson correlation analysis was used to examine the relationship between the growth characteristics of forest and understory vegetation and the WUE and soil, leaf and root C:N:P stoichiometry. Linear regression analysis was used to study the effects of the SQI on the WUE and the plant-soil C:N:P stoichiometry. All indicators used to calculate the SQI were scaled to range between 0 and 1 and plotted using the ggradar package in R. Statistical analyses were performed using SPSS 22.0 software (SPSS Inc., USA), and figures were generated using the R software package (version 4.0.0).

3. Results

3.1. Vegetation and soil characteristics

The R, H, D and E values reached their highest values at 35 years with increasing afforestation age, and the E value showed significant differences among the different afforestation ages ($P < 0.05$) (Table 2). There were significant differences in soil pH, SWC, and SBD among different afforestation ages ($P < 0.01$). The SBD in the 35-year-old stand was significantly lower than that in other stand ages (22.14% − 31.45%) (Table 2). With increasing afforestation age, there was no significant difference in soil texture, but there was a significant difference in the NO_3^--N content among different years ($P < 0.01$), reaching the highest value at 35 years, which was significantly higher than the values of 14.32% − 36.37% calculated for the other years (Table 2).

3.2. Effects of afforestation age on WUE and stoichiometry

With the increase in afforestation age, the WUE reached its highest value in the 35-year-old stand, which was significantly higher than the value of 15.12% in the 8-year-old stand, but there was no significant difference compared with the stands of 15 and 25 years (Fig. 2). The SOC, TN, soil C:P and N:P in the stand of 35 years were significantly higher than those in the other years ($P < 0.01$) (Fig. 3). Soil TP significantly decreased with increasing afforestation age ($P < 0.01$). Soil C:P significantly increased with increasing afforestation age ($P < 0.05$), although there was no statistical significance between some years. The leaf N content of *R. pseudoacacia* in the stands with ages of 8 and 25 was significantly higher than that in the stands with ages of 15 and 35, and the leaf P content of *R. pseudoacacia* in the stands with ages of 8 and 25 was significantly higher than that in the stand with an age of 15 (Fig. 3). The contents of C, N and P in roots at 35 years were the highest. The C content in roots at 35 years was significantly higher than that at 8 years, the N content was significantly higher than that at 8 years and 15 years, and the P content was significantly higher than that at 15 years and 25 years (Fig. 3). In addition, the leaf C content and leaf N:P were not affected by afforestation age ($P = 0.172$ and $P = 0.206$, respectively), and root C:N was not affected by afforestation age ($P = 0.208$) (Fig. 3).

3.3. Linking WUE and plant-soil C:N:P stoichiometry with growth characteristics of forest and understory plants

The WUE and soil, leaf and root C:N:P stoichiometry of *R. pseudoacacia* had different responses to forest growth characteristics and understory species community characteristics (Fig. 4). The WUE was positively correlated with DBH and density. Leaf N and C:N were significantly correlated with DBH, density and CD; leaf P and C:P were significantly correlated only with DBH; and leaf N:P was negatively correlated only with tree height. Root C had a significant positive correlation with DBH and density; root N had a significant positive correlation with tree height, density and E; and root P had a significant positive correlation with R, H and E. Compared with plant stoichiometry, soil stoichiometry was more closely related to the growth characteristics of forest and understory plants. The SOC and soil C:P were significantly positively correlated with the tree height, density, CD, H and E; TN was positively correlated with CD; TP had a significant negative correlation with the tree height, DBH and density; soil C:N had a significant positive correlation with the tree height, density, H and E; and soil N:P had a significant positive correlation with the tree height, density, CD and E.

3.4. Influence of stand quality of R. Pseudoacacia on WUE and plant-soil C:N:P stoichiometry

The results showed that the cumulative contribution of variance of the first three principal components reached 91.65% when these indexes were comprehensively calculated into the SQI. The first three principal components could explain the stand quality information of the *R. pseudoacacia* artificial forest more completely (Table 3). The radar plot indicated that the growth characteristics of *R. pseudoacacia* and understory plants depended on afforestation age (Fig. 6). Specifically,

Table 2
Plant community characteristics and soil properties during succession.

Site	8-year	15-year	25-year	35-year	CV (%)	F	P
Species richness	5.67 ± 0.88a	5.00 ± 1.00a	4.00 ± 1.00a	6.67 ± 1.20a	34.23	1.193	0.372
Shannon-Weiner index	1.09 ± 0.14ab	0.90 ± 0.26b	0.93 ± 0.18b	1.69 ± 0.22a	39.08	3.238	0.082
Simpson diversity index	0.57 ± 0.04ab	0.44 ± 0.12b	0.55 ± 0.06ab	0.78 ± 0.05a	28.94	3.407	0.074
Evenness index	0.64 ± 0.05b	0.57 ± 0.12b	0.70 ± 0.02ab	0.90 ± 0.05a	23.35	4.333	0.043*
Soil pH	8.54 ± 0.04a	8.61 ± 0.06a	8.11 ± 0.04b	8.47 ± 0.06a	2.54	17.325	0.001**
SWC (%)	20.42 ± 0.05b	23.25 ± 0.26a	22.32 ± 0.65a	22.15 ± 0.34a	5.51	9.074	0.006**
SBD (g·cm^{-3})	1.05 ± 0.03b	1.19 ± 0.00a	1.05 ± 0.04b	0.82 ± 0.02c	14.26	30.680	0.000***
Soil sand content (%)	9.22 ± 0.64a	10.65 ± 0.58a	10.14 ± 0.33a	9.77 ± 0.28a	9.03	1.540	0.277
Soil silt content (%)	66.02 ± 0.03a	65.83 ± 0.19a	66.73 ± 0.40a	66.20 ± 0.40a	0.86	1.645	0.255
Soil clay content (%)	24.76 ± 0.61a	23.52 ± 0.65a	23.13 ± 0.07a	24.03 ± 0.28a	3.94	2.249	0.160
AP (mg·kg^{-1})	1.14 ± 0.05a	0.63 ± 0.05b	0.88 ± 0.06ab	1.04 ± 0.41a	29.39	3.380	0.075
NO_3^- (mg·kg^{-1})	3.18 ± 0.11bc	3.50 ± 0.14b	2.93 ± 0.12c	4.00 ± 0.37a	13.37	13.913	0.002**
NH_4^+ (mg·kg^{-1})	20.45 ± 1.20ab	18.83 ± 2.82b	28.82 ± 1.69a	23.62 ± 7.87ab	23.63	3.139	0.087

Note: Data are mean ± standard error. Different letters in the same row indicate significant differences at the 0.05 level among different stand ages. CV is the coefficient of variation. SWC, soil water content; SBD, soil bulk density; AP, Available phosphorus; NO_3^--N, nitrate nitrogen; NH_4^+- N, ammonium nitrogen. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

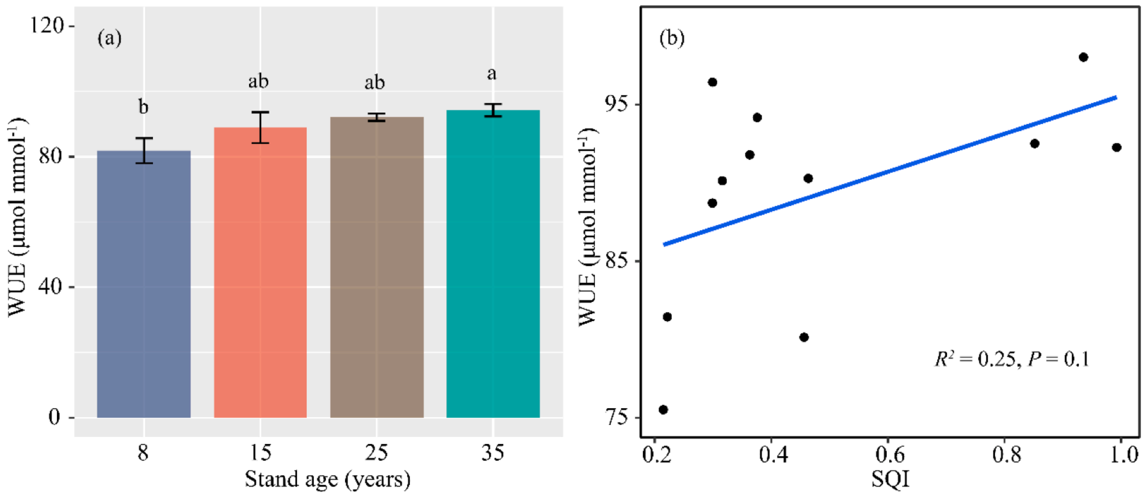

Fig. 2. (a) Water use efficiency (WUE) in each stand age of *Robinia pseudoacacia*. Error bars show the standard error. Different letters indicate significant differences at the 0.05 level ($P < 0.05$) among different stand ages. (b) Effects of stand quality index (SQI) on WUE.

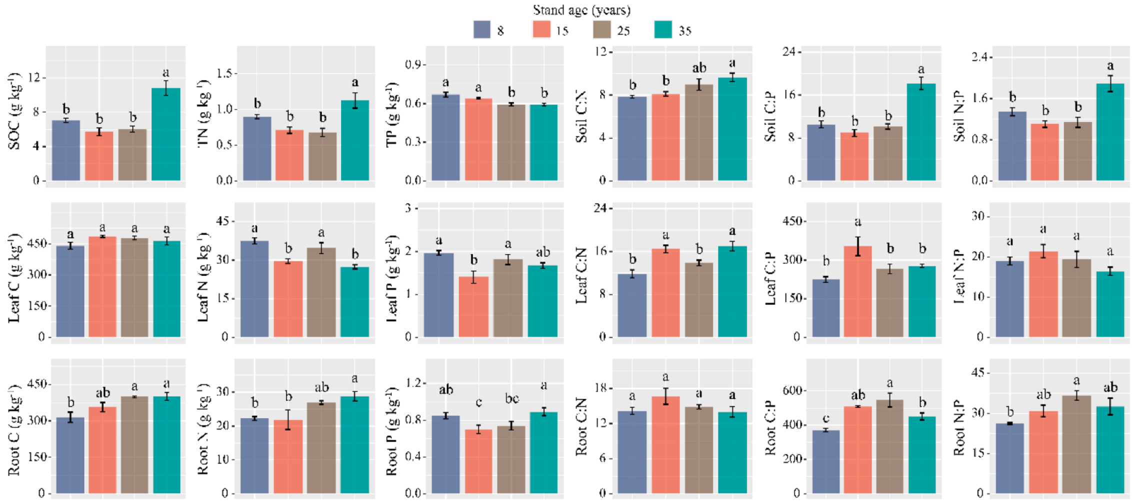

Fig. 3. Changes in soil, leaf and root C, N, and P concentrations and stoichiometry in *Robinia pseudoacacia* at sites with different stand ages. SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus. Error bars show the standard error. Different letters indicate significant differences at the 0.05 level ($P < 0.05$) among different stand ages.

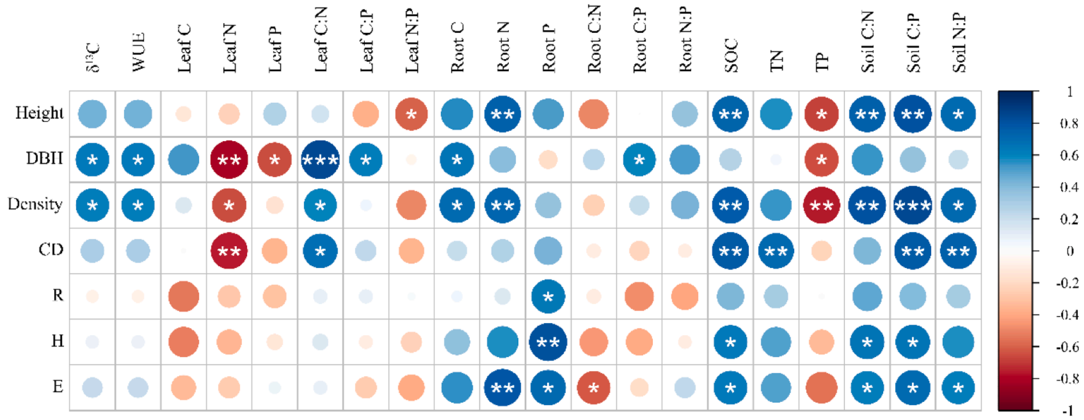

Fig. 4. Pearson correlation coefficients between the forest growth characteristics, understory species community characteristics and WUE and the stoichiometry of soil, leaves and roots of *Robinia pseudoacacia*. The size of the circle indicates the strength of the correlation. Blue circles indicate positive correlations, and red circles indicate negative correlations. DBH, diameter at breast height; CD, canopy density; R, species richness; H, Shannon-Wiener diversity index; E, evenness index; WUE, water use efficiency; C, carbon; N, nitrogen; P, phosphorus. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3
Results of principal component analysis and weight calculation.

Index	Component Matrix			Communalities	Weight
	1	2	3		
Aspect	0.851	−0.150	−0.450	0.949	0.086
Tree height	0.844	0.063	−0.504	0.970	0.099
Diameter at breast height	0.463	0.762	0.391	0.947	0.165
Stand density	0.907	0.398	−0.075	0.986	0.169
Canopy density	0.741	0.173	0.456	0.787	0.165
Species richness	0.551	−0.592	0.520	0.924	0.078
Shannon-Weiner index	0.867	−0.397	0.228	0.961	0.121
Evenness index	0.880	−0.110	−0.144	0.807	0.118

compared with other stand ages, the 35-year-old *R. pseudoacacia* had the highest tree height, DBH, R, H and E, indicating that the stand quality of the 35-year-old stand was higher than that of the other stand ages. Furthermore, the R at 8 years was second only to that at 35 years, the DBH at 15 years was second only to that at 35 years, and the tree height at 25 years was second only to that at 35 years for *R. pseudoacacia*.

WUE and plant and soil stoichiometry had different responses to stand quality. With the increase in the SQI, the WUE had no significant change (P greater than 0.05) (Fig. 2). The leaf N content significantly decreased with increasing SQI ($P < 0.05$) (Fig. 5). The root C and N contents significantly increased with increasing SQI ($P < 0.05$) (Fig. 5). The SOC, TN, soil C:N, C:P and N:P significantly increased with increasing SQI ($P < 0.05$), while the TP significantly decreased with increasing SQI ($P < 0.05$) (Fig. 5).

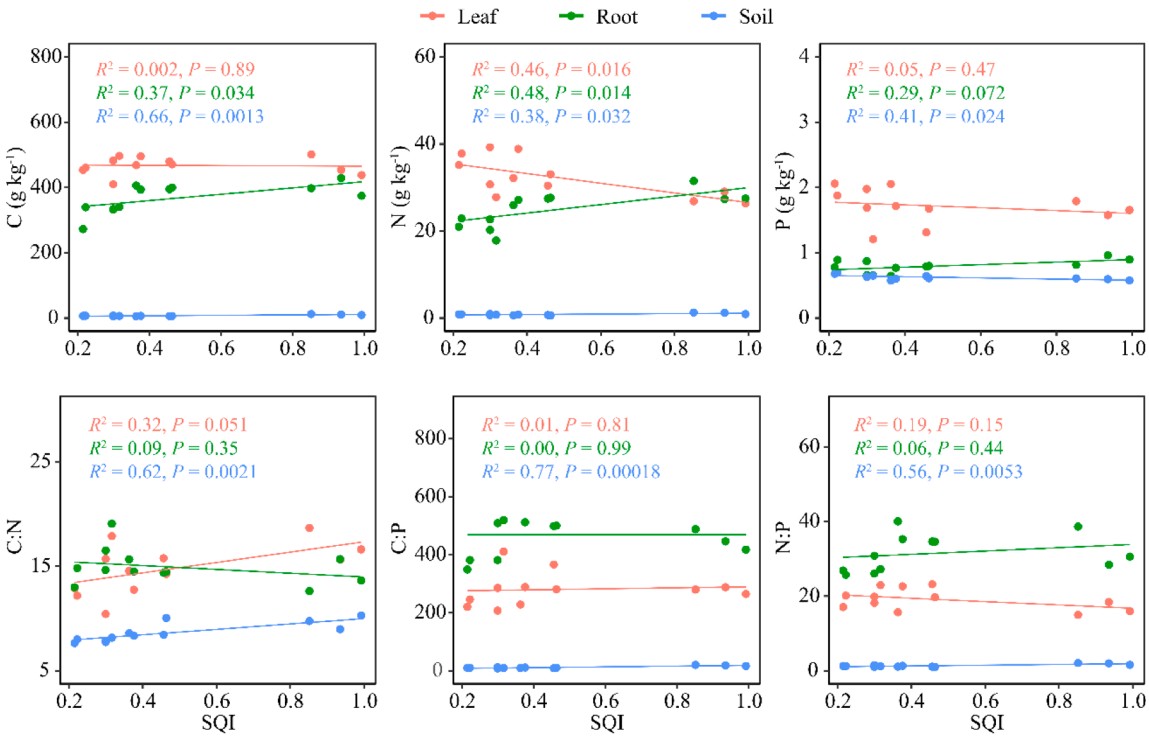

Fig. 5. Relationships between soil, leaf and root C:N:P stoichiometry and the SQI.

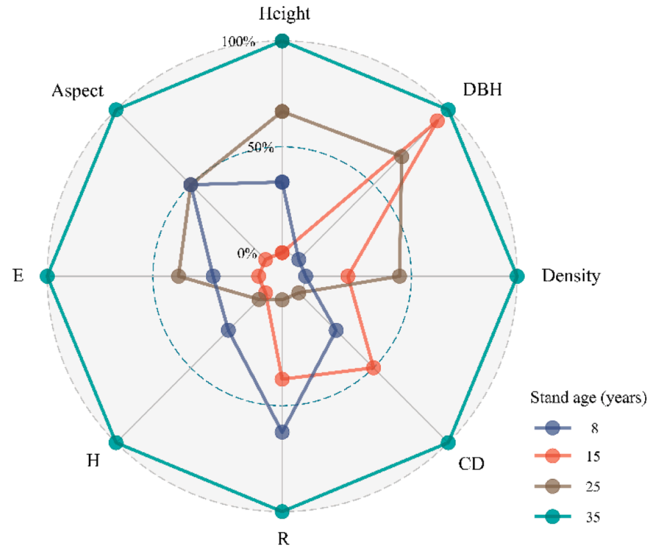

Fig. 6. Radar plots describing the effects of *Robinia pseudoacacia* in different stand ages on different indicators of stand quality. DBH, diameter at breast height; CD, canopy density; R, species richness; H, Shannon-Wiener diversity index; E, evenness index.

4. Discussion

4.1. Changes in plant WUE after afforestation

Plant community succession results in changes in community structure, composition and function; hence, plants in different succession stages have different water use strategies (Raevel et al., 2012), resulting in different plant WUEs. Our results showed that the water stress did not increase significantly with increasing stand age and there was no significant change in WUE with increasing SQI, which was not consistent with our hypothesis. An experiment on the Loess Plateau showed that the leaf WUE of *R. pseudoacacia* decreased significantly with increasing tree height, suggesting that *R. pseudoacacia* may be related to water limitation in semi-arid regions (Tanaka-Oda et al., 2010). WUE is influenced by photosynthetic rate and stomatal conductance. Consequently, the possible reasons for the decrease in WUE are, on the one hand, increased stomatal transpiration and increased cell water loss during photosynthesis (Katul et al., 2010). On the other hand, it may be that with the increase in tree height, leaf size increases and leaf N content decreases, leading to a decrease in the leaf photosynthetic rate (Evans, 1989; Hikosaka, 2004; Katahata et al., 2007). The main reason for the inconsistency between the results of this previous experiment and our current study may be the difference in precipitation at the sampling sites. This previous experiment was conducted in the semi-arid area of the Loess Plateau with the mean annual precipitation is approximately 400 mm, whereas our sampling site was in the semi-humid area of the Loess Plateau with the mean annual precipitation is approximately 584 mm. This difference may suggest that the positive succession of vegetation at a small scale may be determined by local climatic conditions.

During the development of artificial vegetation on the Loess Plateau, excessive water consumption by vegetation growth and transpiration leads to the deficiency of soil water within the scope of plant roots. Large-scale artificial vegetation planting leads to excessive consumption of soil water, which cannot be effectively compensated by natural precipitation for a long time, thus leading to vegetation degradation (Chen et al., 2007; Zhang et al., 2019d). Studies have reported that the difference in soil moisture status is the main factor causing the difference in WUE (Li, 2000; Picotte et al., 2007). Vegetation construction leads to a reduction in SWC, and to reduce water evaporation, the stomatal conductance of leaves decreases and the intercellular CO_2 concentration decreases (Liu et al., 2015). Therefore, with the decrease in soil water content, photosynthate $\delta^{13}C$ increases, increasing plant WUE (Farquhar et al., 1989). However, the relationship between soil water and leaf WUE is maintained within a range, and the correlation will change if the range is higher or lower (Zhang et al., 2019c). This study measured only the SWC data of the 0–20-cm soil layer, and it was found that the root depth of *R. pseudoacacia* could extend to 25 m, allowing the plant to obtain the soil moisture from the deeper soil layer (Wu et al., 2021). To further study the characteristics of water use and water adaptability of *R. pseudoacacia*, future research should combine the long-term monitoring data of plant transpiration, the sap flow of the trunk, the water potential of branches, the root distribution and the deep soil moisture. In addition, our study found that WUE was positively correlated with DBH and density, by combining the relationship of WUE and forest growth characteristics in other studies (Billings et al., 2016; Prasolova et al., 2005; Tanaka-Oda et al., 2010), we infer that WUE may be used to predict and evaluate forest growth potential. Well-grown vegetation played an important role in maintaining WUE, and a reasonable density structure was very important for stand growth. Therefore, regulating the appropriate stand density through tending and thinning would help improve the stand environment, the growth conditions of trees and understory species, and the increase in WUE (Park et al., 2018; Wang et al., 2020).

4.2. Changes in C:N:P stoichiometry in plants and soils after afforestation

In this study, leaf C was not affected by afforestation age, mainly because C in plants is a skeletal element and does not directly participate in plant production activities. However, with increasing afforestation age, the N and P contents of *R. pseudoacacia* leaves decreased, indicating that the photosynthetic rate of mature *R. pseudoacacia* forests decreased, the growth rate slowed, and the competitiveness for nutrient resources needed for growth decreased (Poorter and Bongers, 2006). This is also a possible explanation for the significant decrease in leaf N content with the increase in the SQI. The leaf N:P was not affected by afforestation age, indicating that *R. pseudoacacia* can adapt to environmental changes by adjusting its structure and function, and it can keep the leaf N:P stable, which reflects the homeostasis mechanism of *R. pseudoacacia* plantations to a certain extent (Persson et al., 2010; Yu et al., 2011). Many studies have used the leaf N:P ratio to indicate nutrient limits in an ecosystem. In general, a leaf N:P ratio < 14 indicates an N limitation, a leaf N:P ratio > 16 indicates a P limitation, and a leaf N:P ratio between 14 and 16 indicates that N and P are co-limited (Koerselman and Meuleman, 1996). In this study, the values of leaf N:P indicated that P was the main limiting element in the *R. pseudoacacia* forest, which supported the hypothesis that P in forest soil was increasingly restricted compared with N in previous studies (Fan et al., 2015; Huang et al., 2013; Su and Shangguan, 2020; Wardle et al., 2004b). With increasing afforestation age, the root C and N contents reached their highest values in the stand with an age of 35 years, which resulted in no significant change in the root C:N. The root C and N contents increased significantly with increasing SQI. The increase in root N may be the result of the increase in soil N related to stand age because the root N content reflects soil fertility to some extent (Chen et al., 2018). The C:P ratio of plant tissues can reflect the growth rate of plant tissues; that is, faster-growing tissues require more P-rich ribosomal RNA to support protein synthesis and thus exhibit a lower C:P ratio (Elser et al., 2000; Yuan et al., 2011). The highest P concentration was observed in the roots of 35-year-old *R. pseudoacacia,* and the C:P ratio was lower than that in the 15- and 25-year-old stands, indicating that the 35-year-old *R. pseudoacacia* had a higher fine root growth rate than that in the 15- and 25-year-old stands. This was inconsistent with earlier observations indicating that older *R. pseudoacacia* roots had a higher C:P ratio than younger roots (Cao and Chen, 2017; Chen et al., 2018). Possible explanations for this difference are differences in climatic conditions and age sequence at the sampling sites.

Our results showed that the SOC and TN at 35 years were significantly higher than those at other stand ages, and a similar trend was found in the age series of other afforestation species (Chen et al., 2018; Wang and Zheng, 2021). The main reason for this trend may be that in the process of vegetation restoration, the productivity of vegetation is improved, the decomposition of litter is accelerated, and the quantity and quality of organic compounds are increased (Cao and Chen, 2017; Deng et al., 2016), eventually leading to the accumulation of SOC and TN of *R. pseudoacacia* at 35 years. The TP content of *R. pseudoacacia* decreased with increasing afforestation age, which may be due to the increase in P uptake by plants. In addition, experiments carried out before recorded an increase in soil acid phosphatase activity with the progression of forest succession (Huang et al., 2013), which confirmed that P was the main limiting element for forest growth during afforestation. The above results also explained why SOC and TN increased significantly while TP decreased significantly with the increase in the SQI. The increase in SOC and TN and the decrease in TP led to increases in soil C:P and N:P, and these changes may have been related to the input of more plant litter and roots, which was consistent with the results of other studies (Liu et al., 2020b). Plant litter and roots are important sources of soil C and N. Many experiments on litter decomposition have shown that an increase in plant litter and roots will lead to the accumulation of soil C and N, which is related to the characteristics of litter and roots (Berg, 2014; Berg, 2018).

It has been reported that there is a negative correlation between tree growth and the N:P ratio at both the plant and the leaf levels (Elser et al., 2010; Fan et al., 2015; Gusewell, 2004). Similar results were obtained in this study; that is, there was a significant negative correlation between leaf N:P and tree height. In addition, the results of this study showed that leaf nutrients and stoichiometry were closely related to forest growth characteristics, while root nutrients and stoichiometry were more related to understory plant composition and diversity. This difference may be because understory plants cover the surface layer of soil and have a higher branching capacity of absorptive roots than trees, which can directly transport nutrients for the surface layer of soil (Huang et al., 2016; Jiang et al., 2018), which further affects the nutrient accumulation of tree roots. Some studies have shown that due to the competition of understory plants, the fine root biomass of trees is promoted and increased (Liao et al., 2019), which indirectly supports our results. Moreover, significant correlations were found between soil nutrients and stoichiometry and understory plant composition and diversity. The availability of soil nutrients influence plant establishment and community succession (Bartels and Chen, 2010). Forest succession leads to changes in understory plant community composition and diversity, and different plant community compositions have different abilities to restore soil nutrients and change the microenvironment (Liu et al., 2018; Zhang et al., 2019b). Consequently, soil nutrients are not only the main factors affecting forest growth, but also the key resources affecting the understory plant composition and diversity.

5. Conclusions

Our study showed that stand age had different effects on C, N, and P nutrient contents and stoichiometry in plants and soils. Soil TP decreased significantly with increasing afforestation age, and leaf N:P was greater than 16 at all stand ages, which indicated that P was the main limiting factor in *R. pseudoacacia* forest. The leaf C and N:P and the root C:N of *R. pseudoacacia* did not change with stand age. Moreover, we observed that leaf nutrients and stoichiometry were closely related to forest growth, while root nutrients and stoichiometry were more related to understory plant composition and diversity. In addition, with the increase in the SQI, WUE did not significant change. The influence of stand quality on soil nutrients and stoichiometry was greater than that of plants. These findings provide a framework for the relationship between the growth of artificial *R. pseudoacacia* forests and WUE and C:N:P stoichiometry on the Loess Plateau. Future research is needed to evaluate forest stand growth dynamics and stand productivity, and to explore the potential mechanism of relationships between forest quality and water and nutrient use at a large scale, which could better provide information for how to respond to water and nutrient constraints in the process of vegetation restoration, as well as a theoretical basis for sustainable forest management and forest quality improvement.

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.

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