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Water and nutrient use efficiencies of Robinia pseudoacacia, Amygdalus davidiana, and Armeniaca sibirica and their potential driving forces in monoculture and mixed stands on the Chinese Loess Plateau

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Abstract

Aims The effects of mixed plantations, in comparison with corresponding monoculture plantations, on both water and nutrient use efficiencies remain unclear.

Methods Robinia pseudoacacia mixed (R. pseudoacacia and Amygdalus davidiana, RPAD; R. pseudoacacia and Armeniaca sibirica, RPAS) and monoculture (R. pseudoacacia, RP; A. davidiana, AD; and A. sibirica, AS) stands on the Loess Plateau were used to determine leaf water (WUE), nitrogen (NUE), and phosphorus (PUE) use efficiencies and their potential driving forces.

Results Mixing R. pseudoacacia and A. sibirica is a good strategy to maximize soil nutrient resources. Compared with monocultures of all species, R. pseudoacacia and A. davidiana in RPAD and A. sibirica in RPAS had higher WUE and lower NUE and PUE; P limitation of R. pseudoacacia in either RPAD or RPAS was lower than that in RP. As a whole, a trade-off was observed between WUE and NUE in all species, and between WUE and PUE in A. davidiana and A. sibirica. Moreover, WUE was primarily driven by stand density (SD), crown area (CA), soil total nitrogen (TN), and total phosphorus (TP) for R. pseudoacacia and A. sibirica; SD, CA, and TN for A. davidiana; SD, CA, and TP for A. sibirica. NUE was driven by leaf nitrogen content per mass (N_{mass}), soil organic carbon (SOC), and TP for R. pseudoacacia and A. sibirica, N_{mass} and SOC for A. davidiana.

Conclusions Although mixed species showed different water and nutrient use efficiencies based on inherent factors and site conditions, the trade-off between WUE and NUE remained unchanged.

1 Introduction

The Chinese Loess Plateau (CLP) has an area of about 6.4×10^5 km^2, and the destruction of the original vegetation has resulted in one of the most severe water and soil erosions in the world (Fu et al., 2004). To improve the ecological environment of the CLP, the Grain for Green Project was launched in 1999 (Cao et al., 2011). The plantation coverage on the CLP increased from 31.6% in 1999 to 59.6% in 2013 (Chen *et al.*, 2015). Moreover, the afforestation project changed the cycle of water and nutrients in the plant–soil system (Su and Shangguan, 2021). In general, plant growth is severely constrained by deficient water supply and weak nutrient uptake (Wei et al., 2018). Previous studies used carbon stable isotope ($\delta^{13}C$) analysis and chemical stoichiometry (C:N:P) in plant organs and soil at the stand or regional scale to study water and nutrient status of plants on the CLP (Cao and Chen, 2017; Su and Shangguan, 2020). However, because of the use of various resource acquisition methods, plant growth in this region is characterized by different nutrient use strategies (Bacon, 2004; Gong et al., 2011).

The interspecific relationship between different tree species can be reflected by their water and nutrient use strategies (Luo et al., 2004; Liu et al., 2015). For example, plants respond to water and nutrient stress by changing their physiological activities, such as water (WUE), nitrogen (NUE), and phosphorus (PUE) use efficiencies (Forrester et al., 2005). Generally, plants indirectly reduce their WUE by opening the leaf stomata to increase the intercellular carbon dioxide (CO_2) concentration, photosynthetic rate per unit nitrogen, and transpiration water consumption (Farquhar and Richards, 1984; Garrish et al., 2010). With the increase in soil available nitrogen (AN), leaf nitrogen concentration also frequently increases while NUE decreases, which in turn increases WUE (Ripullone et al., 2004). Guo *et al.* (2016) found that when AN was unchanged, interspecific competition significantly increased the leaf nitrogen concentration but decreased its WUE. In contrast, Garrish et al. (2010) found that WUE of the tropical plant *Ficus insipida* varied as a function of soil AN but not AP. Moreover, according to Dijkstra et al. (2016), WUE increased when water supply was low and nutrient supply was high, whereas NUE showed the exact opposite trend, and physiological constraints generated a trade-off between WUE and NUE. In addition, strong correlations have been observed between PUE and WUE that are determined by the plant genotype (Meier et al., 2022).

As previous studies focused on the changes in WUE and NUE of monoculture stands (Su and Shangguan, 2021), it remains unclear whether mixed-species plantations can attenuate regional water and nutrient stress by increasing both WUE and nutrient use efficiency (i.e., NUE and PUE) of the overall community. A mixed-species plantation is a stand composed of at least two tree species, where the proportion of the main tree species is equal to or greater than 10% (Coll et al., 2018). Because of niche differentiation, positive interactions, or both, the mixed stand can improve its use efficiency of resources, such as light, water, and nutrients, compared with the monoculture stand (Anderegg et al., 2018). For example, mixed stands containing N-fixing species can increase soil total N stocks by biological N fixation and improve the growth of neighboring species in N-limited ecosystems (Taylor et al., 2017). Moreover, the interaction of species with the same growth cycle or leaf phenology or belonging to the same general ecological niche may lead to increased competition for water and nutrients among them (Drossler et al., 2018). However, coexisting species in a community can avoid, to some extent, direct interspecific competition when their differences in root depth and density allow each species to exploit different underground niches, leading to greater and more efficient nutrient consumption (Dimitrakopoulos and Schmid, 2004).

Robinia pseudoacacia (Qiu et al., 2010) is a representative N-fixing species in a wide range of habitats spanning from Europe to China (Shan et al., 2002). On the CLP, this tree species has been planted on an area greater than 7.0×10^4 ha. Unreasonable afforestation measures can cause the ecological degradation of a *R. pseudoacacia* monoculture stand, frequently leading to dead branches and even death (Wei et al., 2018). To guide suitable management and conservation of *R. pseudoacacia*, many studies have explored its water use and nutrient strategies, as well as their driving forces. For example, Tanaka-Oda et al. (2010) found that WUE and N concentration of *R. pseudoacacia* decreased with increasing tree height, indicating that water and nutrients are crucial factors for its growth. Apart from water limitation (Wang et al., 2021), the growth of *R. pseudoacacia* is limited by the availability of P (Cao and Chen, 2017). In addition, previous studies have analyzed the nutrient resorption efficiency of *R. pseudoacacia* monocultures (Qiu *et al.*, 2021). However, few studies have reported water and nutrient use strategies of *R. pseudoacacia* in mixed stands on the CLP. Two common native tree species in this area are *Amygdalus davidiana* and *Armeniaca sibirica*, and they are widely mixed with *R. pseudoacacia*. However, it is unclear whether these mixed patterns can improve the water and nutrient suitability of *R. pseudoacacia*, *A. davidiana*, and *A. sibirica*.

To address this knowledge gap, we analyzed water and nutrient use efficiencies of *R. pseudoacacia* and *A. davidiana* with *A. sibirica* in both monoculture and mixed stands and analyzed their correlations and driving forces. We hypothesize that (1) the mixed stand can increase WUE, NUE, and PUE and alleviate nutrient limitation; (2) there is a trade-off between WUE, NUE, and PUE for these tree species; and (3) WUE, NUE, and PUE are affected by many factors, including stand characteristics and soil properties. The results provide new insights into water and nutrient use efficiencies of mixed afforestation.

2 Materials And Methods

2.1 Study site

The study site is located in Ansai District, Shaanxi Province (36°35'29.35"–36°35' 57.59"N, 109°13'19.84"–109°16'31.85"E, 1195–1212 m above sea level), China. This region belongs to a typical forest–steppe ecotone with a warm temperate semi-humid continental monsoon climate. The mean annual temperature is 8.8°C, and the mean annual precipitation is 500 mm, with most precipitation occurring from July to September. The soils are classified as Calcic Cambisols (FAO, 2020). After 20 years of *R. pseudoacacia* afforestation in this region, the plant has become the leading tree species.

2.2 Experimental design

This study was conducted in five stand types with three replicates (15 plots): mixed stands of *R. pseudoacacia* with *A. davidiana* (RPAD) and with *A. sibirica* (RPAS), and monoculture stands of *R. pseudoacacia* (RP), *A. davidiana* (AD), and *A. sibirica* (AS). These plots share similar altitude, slope, soil condition, stand age (16–25 years), and climatic conditions (Table 1). For each stand type, three 20 m × 20 m quadrats were used for the plot survey, which included calculating stand density (SD), average tree height (AH), height under the stem (HB), and diameter at breast height (DBH). To avoid edge effects, a buffer zone of 25 m was established around each plot and the distance between two plots did not exceed 3 km (Figure 1).

2.3 Plot survey and sampling

In August 2021, green leaves from each plot were sampled from lower and middle canopies of five model trees with similar height and DBH and then divided into two parts. One part was placed in an ice box and brought back to the laboratory to analyze leaf functional traits. The other was heated at 105°C for 15 min and then heated to a constant weight at 70°C in an oven to analyze leaf chemical components. To analyze soil chemical properties, 0–20 cm soil was sampled using a soil drill (40 mm in diameter). Soil samples of mixed stands were composed of soil collected near *R. pseudoacacia* and *A. davidiana*, and *R. pseudoacacia* and *A. sibirica* in mixed stands. To analyze soil physical properties, undisturbed soil was sampled using a ring cutter (volume 100 cm^3) at 0–20 cm as above.

2.4 Sample measurement

Five to ten intact, disease-free leaves of the three tree species were sampled in monoculture and mixed stands to measure and calculate leaf functional traits (the method is described in Table 2).

Soil particle composition was determined using a Mastersizer 2000 Laser Particle Size Analyzer (Malvern Panalytical, Malvern, UK). Soil conductivity (EC) was measured using a conductivity meter (DDSJ-308F; INESA Scientific Instrument Co., Ltd., Shanghai, China). Soil bulk density (BD) was determined by the ring knife method (Bao, 2000), whereas soil water content (SWC) was determined by the drying method (Bao, 2000). In addition, 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.

Soil organic carbon (SOC) concentration was determined by the potassium dichromate volumetric method (Nelson and Sommers, 1982). In addition, soil total nitrogen (TN) concentration was determined by the Kjeldahl method (Bremner and Mulvaney, 1982), while soil available nitrogen (AN) concentration was determined by the alkaline hydrolysis diffusion method (Bao, 2000). Finally, both soil total phosphorus (TP) and available phosphorus (AP) concentrations were determined by the molybdenum blue method (Murphy and Riley, 1962).

The stable carbon isotope value $\delta^{13}C$ (‰) was determined by isotope mass spectrometry (MAT 253; Thermo Fisher Scientific, Waltham, MA, USA) using the equation:

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{standard}} \right) \times 1000, \quad (1)$$

where R_{sample} and $R_{standard}$ are the carbon isotope ratio ($^{13}C/^{12}C$) of the samples and the international carbon isotope standard (Pee Dee Belemnite (PDB), R_{PDB} = 0.0112372, $\delta^{13}C_{PDB}$ = 0‰), respectively.

2.5 Calculation of WUE, NUE, and PUE

There is a significant correlation between leaf $\delta^{13}C$ and the ratio of intercellular CO_2 concentration (C_i) to atmospheric CO_2 concentration (C_a, http://www.esrl.noaa.gov) (Farquhar, 1982), and the equation is as follows:

$$\Delta^{13}C(‰) = \delta^{13}C_a - \delta^{13}C = a + (b-a)\frac{C_i}{C_a}, \quad (2)$$

where $\Delta^{13}C$ is the carbon isotope discrimination during photosynthesis, $\delta^{13}C_a$ (−8‰) is the ^{13}C abundance in the atmosphere, and *a* (4.4‰) and *b* (27‰) are the stable carbon isotope fractionation produced by the diffusion and carboxylation reactions, respectively (Farquhar and Richards, 1984).

The leaf conductance to water vapor (gH_2O) is 1.6 times the CO_2 conductance rate (gCO_2), and the net photosynthetic rate (A) is significantly correlated with gH_2O (Penuelas *et al.*, 2011). Thus, A can be calculated with the following equation:

$$A = g_{CO2} \times (C_a - C_i) \quad (3)$$

$\Delta^{13}C$ can be related to A/g_{H2O}, which is long-term water use efficiency (WUE), (Ehleringer, 2011) as follows:

$$WUE = \frac{A}{g_{H2O}} = \frac{C_a - C_i}{1.6} = \frac{b - \Delta^{13}C}{1.6(b-a)} \quad (4)$$

The green leaf C:N and C:P mass ratios were used as a measure of NUE and PUE, respectively (Vitousek, 1982; Dijkstra *et al.*, 2016). The green leaf N:P mass ratio was used to determine plant nutrient limitation (Güsewell, *et al.*, 2004); an N:P ratio less than 14, between 14 and 16, and greater than 16 indicates N limitation, N and P colimitation, and P limitation, respectively (Koerselman and Meuleman, 1996).

2.6 Statistical analysis

The leaf C:N and C:P ratios were calculated from the leaf C, TN, and TP concentrations. Statistical analysis was performed using SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA) at a significance level of 0.05. The data were transformed to meet the assumptions of normality and homogeneity when needed. One-way analysis of variance (ANOVA) and *t*-test were used to analyze the differences in WUE, NUE, PUE, and N:P ratio for the same species in different plantations. Two-way ANOVA was performed with Duncan's multiple range test to verify the effects of tree species, plantation type, and their interaction on WUE, NUE, PUE, and N:P ratio. Pearson correlations and partial correlation coefficients were used to quantify the correlations among WUE, NUE, PUE, N:P ratio, and their potential driving forces. Principal component analysis (PCA) was performed to identify the primary axes of covariation among the driving forces. A regression analysis was performed to examine the relationship between WUE and nutrient use efficiency (i.e., NUE, PUE, and N:P ratio), and between NUE and PUE. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

3 Results

3.1 Factors that modulate WUE, NUE, and PUE

Leaf nitrogen content per unit area (N_{area}) and leaf phosphorus content per unit area (P_{area}) were higher, and DBH, AH, and specific leaf area (SLA) were lower for *R. pseudoacacia* growing in mixed stands than they were for *R. pseudoacacia* growing in the monoculture stand (Table 3). Crown area (CA), LA, SLA, leaf nitrogen content per mass (N_{mass}), and leaf phosphorus content per mass (P_{mass}) were higher, and SD, LT, leaf dry matter content (LDMC), LD, N_{area}, and P_{area} were lower for *A. davidiana* growing in mixed stands than they were for the same species growing in the monoculture stand. AH, CA, LT, SLA, N_{area}, N_{mass}, and P_{mass} were higher, and SD, LDMC, and LD were lower for *A. sibirica* growing in mixed stands than they were for *A. davidiana* growing in monoculture stands.

The values of SWC, EC, BD, SA, and soil clay content (CL) were higher in the RPAD stand than in RP and AD stands, whereas their values in the RPAS stand were between those in RP and AS stands (Table 4). Soil pH was lower in the RPAD stand than in RP and AD stands, and it was lower in the RPAS stand than in RP and AS stands. The soil TN value in the RPAD stand was between that in RP and AD stands, and it was between that in RP and AS stands in the RPAS stand. The values of AN and AP were lower in the RPAS stand than in RP and AS stands, whereas their values in the RPAD stand were between those in RP and AD stands.

PCA results of the driving forces of WUE, NUE, and PUE are shown in Figure 2. PC1 explained 39.5% of the total variance and the load mainly from LT, P_{area}, LDMC, CA, SD, N_{mass}, P_{mass}, AH, HB, SLA, and EC ($P < 0.05$), while PC2 explained 22.4% of the total variance and the load mainly from BD, AN, TN, and SOC ($P < 0.05$). In addition, the dispersion within the same species depending on the community was lower in *R. pseudoacacia* than in the other two native non-N-fixing species.

3.2 WUE, NUE, and PUE

Both tree species and plantation type had a significant impact on WUE, NUE, and PUE (Table 5). Compared with the RP stand, WUE of *R. pseudoacacia* was higher in the RPAD stand ($P < 0.05$), and NUE of *R. pseudoacacia* ($P < 0.05$) was higher in the RPAS stand (Figure 3a, b). Similarly, the values of WUE of *A. davidiana* and *A. sibirica* in mixed stands were higher ($P < 0.05$) than they were in monoculture stands (Figure 3b), and the values of PUE of *A. davidiana* and *A. sibirica* in monoculture stands were higher ($P < 0.05$) than in mixed stands (Figure 3c). Compared with the RP stand, the N:P ratio of *R. pseudoacacia* was lower in the RPAS stand, whereas that of *A. davidiana* was significantly higher in the RPAD stand (Figure 3d).

WUE of *R. pseudoacacia* and *A. davidiana* in the RPAD stand and that of *A. sibirica* in the RPAS stand was 17.47%, 13.99%, and 5.83% higher than that of tree species in the monoculture stands, respectively. NUE of *R. pseudoacacia* and *A. davidiana* in the RPAD stand and *A. sibirica* in the RPAS stand was 2.78%, 30.65%, and 26.34% lower, respectively; whereas NUE of *R. pseudoacacia* in the RPAS stand was 8.23% higher, and PUE of *A. davidiana* in the RPAD stand and *A. sibirica* in the RPAS stand was 27.48% and 18.34% lower than that of tree species in the monoculture stands, respectively (Figure 4).

3.3 Correlations among WUE, NUE, PUE, and leaf N:P ratio

On the whole, there were negative correlations between WUE and NUE of *R. pseudoacacia* ($P < 0.05$), *A. davidiana* ($P < 0.05$), and *A. sibirica* ($P < 0.01$; Figure 5a, b, c; Figure 6a), and between WUE and PUE of *A. davidiana* ($P < 0.01$) and *A. sibirica* ($P < 0.01$; Figure 5b, c; Figure 6b). Except for *R. pseudoacacia* and *A. sibirica*, WUE was negatively correlated with the N:P ratio for *A. davidiana* ($P < 0.05$; Figure 5b, c; Figure 6c).

In addition, NUE was positively correlated with PUE for *A. davidiana* ($P < 0.05$) and *A. sibirica* ($P < 0.01$; Figure 5b, c; Figure 6d). The N:P ratio was negatively correlated with NUE for *R. pseudoacacia* ($P < 0.05$) and *A. sibirica* ($P < 0.05$; Figure 5a, c; Figure 6e). However, PUE was negatively correlated with the N:P ratio for *A. sibirica* ($P < 0.05$; Figure 5a, c; Figure 6e).

3.4 Correlations among WUE, NUE, and PUE, with their driving forces

In both monoculture and mixed stands, WUE was positively correlated with SD in *R. pseudoacacia* ($P < 0.05$; Figure 7a), whereas it was the opposite in *A. davidiana* and *A. sibirica* ($P < 0.05$; Figure 7b, c). NUE was negatively correlated with N_{mass} in *R. pseudoacacia*, *A. davidiana*, and *A. sibirica* ($P < 0.05$; Figure 7a, b, c). In *A. davidiana* and *A. sibirica*, the PUE values were positively correlated with SD, LDMC, and LD and negatively correlated with AH, CA, HB, LT, N_{area}, N_{mass}, and P_{mass} ($P < 0.05$, Figure; 7b, c). The N:P ratio was positively correlated with N_{mass} in *R. pseudoacacia* and *A. sibirica* ($P < 0.05$; Figure 7a, c).

WUE was negatively correlated with TN and TP in *R. pseudoacacia* ($P < 0.05$; Figure 7a), whereas it was positively correlated with TN in *A. davidiana* ($P < 0.05$; Figure 7b) and TP in *A. sibirica* ($P < 0.05$; Figure 7c). NUE was positively correlated with TP and SOC in *R. pseudoacacia* ($P < 0.05$; Figure 7a), whereas it was negatively correlated with SOC in *A. davidiana* ($P < 0.05$; Figure 7b), and it was positively correlated with SOC and negatively correlated with TP in *A. sibirica* ($P < 0.05$; Figure 7c). The N:P ratio was positively correlated with AP and negatively correlated with SOC in *R. pseudoacacia* ($P < 0.05$; Figure 7a), whereas it was positively correlated with BD in *A. davidiana* and with TP, SWC, and EC in *A. sibirica* ($P < 0.05$; Figure 7b, c).

Combining the partial correlation coefficients of all potential driving forces of WUE, NUE, and PUE showed that SD and N_{area} had the greatest effect on WUE, NUE, and PUE, whereas DBH, AH, and LT had the greatest effect on the N:P ratio (Table 6). In addition, AP had the greatest effect on NUE, PUE, and the N:P ratio (Table 6).

4 Discussion

4.1 Potential driving forces of WUE, NUE, and PUE

The N:P ratio is considered a reliable indicator of nutrient limitation (Güsewell *et al*., 2004), and a leaf N:P ratio (mass basis) less than 14, between 14 and 16, and greater than 16 indicates N limitation, N and P colimitation, and P limitation, respectively (Koerselman and Meuleman, 1996). In our study, according to this criterion, in monoculture and mixed stands, the growth of *R. pseudoacacia* with two native species was limited by P availability (Cao and Chen, 2017), and the growth of *A. sibirica* was limited by N availability (Figure 3d). In addition, the N:P ratio of *R. pseudoacacia* was significantly lower in the RPAS stand, whereas that of *A. davidiana* was significantly higher in the RPAD stand. Therefore, mixed stands decreased the P limitation in *R. pseudoacacia* and the N limitation in *A. sibirica*. In addition, the N:P ratio of *R. pseudoacacia* and *A. sibirica* was significantly positively correlated with N_{mass} and soil AP (Figure 7a, b; Table 6), probably because the processing of N-fixing of rhizobia promotes soil TP transformation to soil AP (Su and Shangguan, 2020).

The results show that the dispersion in multifactorial PCA within the same species depending on the community was lower in *R. pseudoacacia* than in the other two native non-N-fixing species (Figure 2), indicating that the N-fixing capacity of *R. pseudoacacia* confers it with a partial independence from other biotic or abiotic factors, giving it some competitive advantages over non-N-fixing species. Generally, the mixed stand structure directly affects the morphological, physiological, and composition features of its species by influencing the available resources through canopy closure and root distribution (Forrester *et al*., 2006; Dawud *et al*., 2016). In addition, compared with monoculture, the mixed-species plantation structure may improve plantation productivity by increased resource acquisition capability while reducing competition because of some level of niche differences (Danescu *et al*., 2016; Coll *et al*., 2018). Therefore, N_{area} and P_{area} were higher and DBH, AH, and SLA were lower for *R. pseudoacacia* growing in mixed stands than they were for the same species growing in monoculture stands (Table 3).

Mixed species with different root depth and density can exploit various underground niches, leading to more nutrient consumption (Dimitrakopoulos and Schmid, 2004; Stubbs and Wilson, 2004). In our study, AN and AP were lower for *R. pseudoacacia* growing in the RPAS stand than for the same species growing in RP and AS stands, while AN and AP of *R. pseudoacacia* growing in the RPAD stand were between those for the same species growing in RP and AD stands (Table 4). Therefore, *R. pseudoacacia* and *A. sibirica* in the RPAS stand showed more nutrient consumption and better nutrient utilization capacity when growing in the mixed community than when growing in their respective stands. In contrast, this was not observed in mixed stands of *R. pseudoacacia* and *A. davidiana* in the RPAD stand. Thus, the results strongly suggest that mixing *R. pseudoacacia* and *A. sibirica* is an effective strategy to maximize soil nutrient resources on the CLP.

4.2 WUE and correlations with its driving forces

4.2.1 Effect of mixed stand on WUE

Under water-deficit conditions, a high WUE was an important trait for plant survival and growth (Gong *et al.*, 2011). In this study, the WUE values of *R. pseudoacacia* in the RP stand and *A. sibirica* in the AS stand were higher than those of *A. davidiana* in the AD stand (Figure 3a, Figure 4). Studies showed that stomatal conductance decreases with increasing water stress, resulting in an increase in WUE (Liu *et al.*, 2015). Thus, *R. pseudoacacia* and *A. sibirica* show stronger competition and ecological adaptability than *A. davidiana* for water stress growing in monoculture stands under these environmental conditions.

Generally, the success of mixed stands is related to functional complementarity and mutual promotion relationships among multiple tree species (Forrester *et al.*, 2005). In this study, such relationships were not detected with unchanged WUE in species in mixed stands in some cases (Figure 3a, Figure 4), which is in agreement with previous studies and consistent with the observation that plant WUE was associated with functional traits linked to genetic conditions (Meier *et al.*, 2022), such as leaf photosynthetic rate, stomatal conductance, and life cycle (Garrish *et al.*, 2010, Su and Shangguan, 2020). However, mixed afforestation increased WUE of *R. pseudoacacia* when this species was grown along with *A. davidiana* in the RPAD stand by decreasing stomatal transpiration in photosynthesis (Figure 3a, Figure 4; Katul *et al.*, 2010). Specifically, mixed species increase leaf area and decrease leaf N content per unit area as the canopy gradually closes (Table 3), leading to a decreased light-saturated net photosynthetic rate (Katahata *et al.*, 2007).

In this study, WUE of *A. sibirica* growing in the RPAS stand was lower than that of *A. sibirica* growing in the AS stand (Figure 3a, Figure 4). This result strongly suggests that *A. sibirica* in the RPAS stand was under lower drought stress than the same species growing in the monoculture stand. Therefore, *A. sibirica* in the RPAS stand decreased its WUE and increased its use efficiency of other resources, such as light and nutrients (Wu *et al.*, 2017). In contrast, there was no significant change in WUE of *R. pseudoacacia*, whether it was grown in the RPAS stand or in the RP stand (Figure 3a, Figure 4). The mixed-species plantation is divided by hydrological niches, which decrease the competition among species for limited water resources and strengthen regional drought resistance (Lebourgeois *et al.*, 2013; Pretzsch *et al.*, 2013). Therefore, tree species can adopt different water use strategies in mixed stands compared with monoculture stands.

4.2.2 Influence of potential driving forces on WUE

In arid or semi-arid regions, the significant increase in WUE can be attributed to the decrease in stomatal conductance as temperature increased or soil moisture decreased (Jia *et al.*, 2022). When soil water stores gradually deplete (Lei *et al.*, 2022), *R. pseudoacacia* can improve WUE with tight stomatal control and limited transpiration rates (Fu *et al.*, 2020). Therefore, *R. pseudoacacia* monoculture with higher SD tends to expend more soil water stores relative to the same monoculture with lower SD (Tanaka-Oda *et al.*, 2010). In addition, the larger the SD, the smaller the CA of individual trees. This explains the observation that WUE of *R. pseudoacacia* was positively correlated with SD but negatively correlated with CA (Figure 7a, Table 6; Brookshire *et al.*, 2020). Moreover, mixed stands with higher SD and greater canopy closure but smaller tree growth experience lesser photosynthetically active radiation than taller trees, leading to the low temperature inside the plantation (Khanna, 2008; Nygren and Leblanc, 2015). However, WUE of *A. davidiana* and *A. sibirica* in mixed stands was positively correlated with SD and negatively correlated with CA (Figure 7b, c), strongly suggesting that the stand temperature is a dominant factor controlling WUE of understory plants.

With the increase in soil nutrients, plants tend to increase leaf nutrient concentration, reducing nutrient use efficiency, which in turn increase WUE (Ripullone *et al.*, 2004). Furthermore, WUE of *A. davidiana* and *A. sibirica* was positively correlated with soil TN and TP, respectively (Figure 7b, c), which may be related to plant physiology. For example, sufficient soil nutrients can stimulate plants to improve leaf photosynthesis and accumulate biomass, while their effect on evapotranspiration was not significant (Patterson *et al.*, 1997). Thus, the increase in leaf N and P concentrations is generally associated with an enhancement in mesophyll conductance and photosynthetic capacity (i.e., A) (Wright *et al.*, 2004). Similar results were obtained in *Quercus velutina* (Jennings *et al.*, 2016) and *Larix principis-rupprechtii* (Yan *et al.*, 2022). In contrast, *R. pseudoacacia* exhibits an opposite trend than expected because higher soil nutrients imply a lower, rather than a higher, WUE (Figure 7a). One plausible explanation is that N-fixing bacteria increase the N source of *R. pseudoacacia*, causing its fine roots to absorb more water to transport N. As the body stored more water, WUE of *R. pseudoacacia* was decreased and then showed negative correlations with soil TN. In addition, along with absorbing N compounds, the fine roots absorb a large amount of soil P. Therefore, WUE of *R. pseudoacacia* was negatively correlated with soil TP. Further, the decreased rate of soil TP was greater than that of soil TN, which may be another reason for the increased P limitation of *R. pseudoacacia*. All in all, as an N-fixing plant, *R. pseudoacacia* was not dependent on soil N sources, and thus, WUE may be directly related with its capacity to fix N.

4.3 NUE, PUE, and their correlations with driving forces

In monoculture stands, NUE of *R. pseudoacacia* was lower than that of *A. davidiana* and *A. sibirica* (Figure 3b), but AN of *R. pseudoacacia* was not the largest of the three (Table 4), indicating that AN was not necessarily correlated with NUE (Dijkstra *et al.*, 2015). The possible reason was that the growth of *R. pseudoacacia* was at least partially related to atmospheric N fixed by symbiotic N-fixing bacteria (Turner and Lambert, 2014; Nygren and Leblanc, 2015) and thus partially independent of soil N. In addition, litter decomposition of *R. pseudoacacia* can continuously increase AN (Table 4) (Voigtlaender *et al.*, 2012). Furthermore, NUE of these tree species was significantly negatively correlated with soil TP and AP (Table 6), and NUE of *R. pseudoacacia* was lower than that of *A. davidiana* and *A. sibirica* (Figure 3b, Figure 4). Therefore, monoculture or mixed stands, including *R. pseudoacacia,* had higher TP and AP concentrations (Table 4). However, the fact that AP was the lowest in the RPAS stand (Table 4) was probably associated with the observed high water demand for *R. pseudoacacia* and *A. sibirica* in the RPAS stand, promoting the transport of water and nutrients in soil and plant root uptake (Du *et al.*, 2011; Sardans and Penuelas, 2012). Moreover, PUE of *R. pseudoacacia* was the lowest (Figure 3c) and its AP was the largest (Table 4) in monoculture stands, which were in agreement with previous studies that found plants have lower nutrient use efficiency when they grow in nutrient-rich soil (Vitousek, 2010; Turner and Lambert, 2014).

In both monoculture and mixed stands, these tree species maintained similar NUE and PUE (Figure 3b, c). Therefore, their nutrient use strategies are mostly influenced by inherent but not external factors (Deng *et al*., 2019). However, the relative increase in NUE of *R. pseudoacacia* in the RPAS stand was higher than that of *R. pseudoacacia* growing in the RPAD stand (Figure 3b, Figure 4). In addition, TN and AN values of this species were larger in the RPAS stand than in the RPAD stand (Table 4). Therefore, mixing *R. pseudoacacia* and *A. sibirica* should better increase soil N content of the RPAS stand. The reason may be that root growth in *A. sibirica*, due to its characteristics, can increase soil aeration, which indirectly increases and facilitates the symbiotic N-fixation of rhizobia. Otherwise, mixed afforestation did not alter PUE of *R. pseudoacacia* (Figure 3c, Figure 4), which was consistent with previous studies that found mixtures of N-fixing species, including *Albizia falcataria* and *Eucalyptus saligna*, had no effect on the processes of soil P transformation compared with their monocultures (Zou *et al*., 1995). In our study, PUE of *A. davidiana* and *A. sibirica* decreased after mixed afforestation (Figure 3c, Figure 4), whereas their AP was lower in both RPAD and RPAS stands than in RP and AD stands (Table 4). Hence, PUE of these tree species was significantly negatively correlated with their AP (Table 6), which was in line with previous studies that found having mixed-species plantations with the same growth cycle or leaf phenology usually leads to increased competition for resources (Feller *et al*., 1999; Amazonas *et al*., 2018). Moreover, in the short–medium term when these two species grow with *R. pseudoacacia*, they could dispose of more P by the effect of *R. pseudoacacia* allocating resources to increase P mineralization and leaching; thus, as P availability increases, PUE of *A. davidiana* and *A. sibirica* decreases when these species grow along with *R. pseudoacacia*. For example, de-Dios-García *et al.* (2018) showed that mixed forest stands formed only by conifers may show higher competition for resources than conifer-broadleaved admixtures because of more similar plant characteristics. Therefore, these tree species in the RPAD or RPAS stand adopt different nutrient use strategies.

4.4 Trade-off between WUE and NUE, PUE, and N:P ratio

Generally, WUE is negatively correlated with NUE and PUE, because a high WUE is possible only if the photosynthetic machinery and energy transfer capacity is high, which requires high N and P concentrations or low NUE and PUE (Zhao *et al*., 2015; Dijkstra *et al*., 2016). Moreover, as an indirect effect, inefficient WUE can be associated with higher transpiration fluxes, favoring P and overall N (more soluble) uptake, as WUE was inversely related with NUE and PUE, that is, lower WUE was associated with higher NUE and PUE. In this study, we observed a negative relationship between WUE and NUE (Figure 6a), which is in line with previous studies (Gong *et al*., 2011; Dijkstra *et al*., 2016) that found the relationship was consistent with a direct trade-off effect, and the correlation coefficients (R^2) in the increasing order were as follows: 0.55 for *R. pseudoacacia*, 0.80 for *A. davidiana*, and 0.95 for *A. sibirica*. This effect could be due to changes in physiological traits (such as leaf water potential and gas exchange parameter) and study sites with a heterogeneous environment (Yan *et al*., 2016). For example, air temperature and humidity can affect water depletion of plants, which in turn affects their evapotranspiration and ultimately leads to different WUE (Garrish *et al*., 2010). Furthermore, the processing of symbiotic N-fixation can alter root ability in absorbing water and utilizing N (Su and Shangguan, 2020).

N and P are two important limiting resources for plant growth, and both significantly affect WUE (Huang *et al*., 2015). However, few studies have confirmed a stable trade-off between WUE and PUE (Brown *et al*., 2011, Huang *et al*., 2015). This is because N and overall P uptake depend on variables other than soil water movement and transpiration, such as soil enzyme activities, root system form and size, and root exudates. However, for N-fixing species, water uptake and N and P uptake capacities are more disconnected, as these plants can fix N independently after soil uptake and can mobilize more soil P because of their higher capacity of promoting phosphatase synthesis in the plant–soil system by a better supply of N and thus better protein production capacity. This strongly explains why PUE of *R. pseudoacacia* was not correlated with its WUE (Figure 6b). A similar result was reported for *Pinus pinaster* in southwest Western Australia (Warren *et al*., 2005). However, WUE of *A. davidiana* and *A. sibirica* was significantly negatively correlated with PUE (Figure 6b), which is in agreement with a previous study (Zhou *et al*., 2016). This result was consistent with the high water demand of these species in mixed stands along with their higher capacity for P uptake. For example, these species have a greater capacity of phosphatase synthesis by a higher availability of N and thus of protein synthesis because of the N-fixing capacity, which altogether promote the available nutrients (e.g., AP) to the root by mass flow and reduced availability of soil nutrients in the rhizosphere (Cregger *et al*., 2014).

WUE of *R. pseudoacacia* was not positively correlated with the N:P ratio, as other studies found in the Qinghai–Tibet Plateau and the Xilingol grassland (Zhou *et al*., 2016). However, WUE of *A. davidiana* was negatively correlated with the N:P ratio (Figure 6c). Leaf P concentration was positively correlated with the photosynthetic rate (Güsewell, 2004). When the leaf N:P ratio was greater than 12, plant WUE negatively correlated with the photosynthetic rate (Garrish *et al*., 2010). Therefore, plant WUE is affected by the combined action of leaf N and P (Huang *et al*., 2015). In this study, WUE of *A. sibirica* was significantly positively correlated with the leaf N:P ratio (Figure 6c), which was consistent with a previous report in shrubs and trees (Yan *et al*., 2016), and it was also closely linked to the positive correlations between plant N:P stoichiometry and transpiration rate (Cernusak *et al*., 2007), thereby integrating the nutrient and hydrological cycles (Dijkstra *et al*., 2016; Su and Shangguan, 2020). Therefore, this relationship may be affected by multiple factors such as genetic traits and nutrient conditions.

5 Conclusions

In comparison with corresponding monoculture stands, mixing *R. pseudoacacia* and *A. sibirica* was a good strategy to maximize soil nutrient resources. *R. pseudoacacia* and *A. davidiana* in the RPAD stand and *A. sibirica* in the RPAS stand had higher WUE and lower NUE and PUE than they had in monoculture stands. Mixed afforestation decreased the P limitation in *R. pseudoacacia* and the N limitation in *A. sibirica.* A trade-off was observed between WUE and NUE in all species, and between WUE and PUE in *A. davidiana* and *A. sibirica*. WUE of the different tree species was mostly related to their inherent traits, whereas their NUE and PUE were more influenced by soil nutrient factors.

Declarations

Supplementary materials

Non

Availability of data and material

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

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Author contributions

Y.M. Chen and S.B. Lu conceived and designed the study; S.B. conducted the experiment; S.B., Y.M., J.S., and J.P analyzed the results, wrote and edited the manuscript.

Declaration of interests

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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References

1. Amazonas, N.T., Forrester, D.I., Oliveira, R.S., Brancalion, P.H.S.. 2018. Combining Eucalyptus wood production with the recovery of native tree diversity in mixed plantings: Implications for water use and availability. For. Ecol. Manage. 418, 34-40.
2. Anderegg, W.R.L., Konings, A.G., Trugman, A.T., Yu, K., Bowling, D.R., Gabbitas, R., Karp, D.S., Pacala, S., Sperry, J.S., Sulman, B.N., Zenes, N.. 2018. Hydraulic diversity of forests regulates ecosystem resilience during drought. Nature 561 (7724), 538-541.
3. Bacon, M.A.. 2004. Water use efficiency in plant biology. Oxford, UK: Blackwell Publishing, 1-27.
4. Bao, S.. 2000. Soil and agriculture chenistry analysis, third ed. Beijing: China Agriculture Press. (in Chinese)
5. Bremner, J.M., Mulvaney, C.S.. 1982. Nitrogen – total. In: In: Page, A.L. (Ed.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties, Agronomy Monograph, vol. 9 ASA and SSSA, Madison.
6. Brookshire, E.N.J, Stoy, P.C, Currey, B., Finney, B.. 2020. The greening of the Northern Great Plains and its biogeochemical precursors. Global Change Biology 26(10), 5404-5413.
7. Brown, K.R., Courtin, P.J., Negrave, R.W.. 2011. Growth, foliar nutrition and $\delta^{13}C$ responses of red alder (*Alnus rubra*) to phosphorus additions soon after planting on moist sites. For Ecol Manag. 262, 791-802.
8. Cao, S.-X., Chen, L., Shankman, D., Wang, C.-M., Wang, X.-B., Zhang, H.. 2011. Excessive reliance on afforestation in China's arid and semiarid regions: Lessons in ecological restoration. Earth-Sci. Rev. 104, 240-245.
9. 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. 31, 1-12.
10. Cernusak, L.A., Winter, K., Aranda, J., Turner, B.L., Marshall, J.D.. 2007. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. J.Exp. Bot. 58, 3549-3566.
11. Cernusak, L.A., Winter, K., Turner, B.L.. 2010. Leaf nitrogen to phosphorus ratios of tropical trees: experimental assessment of physiological and environmental controls. New Phytol. 185, 770-779.
12. Chen, Y.. 2015. Balancing green and grain trade. NATURE GEOSCIENCE 10(8), 739-741.
13. Coll, L., Ameztegui, A., Collet, C. , Lof, M., Mason, B., Pach, M., et al. 2018. Knowledge gaps about mixed forests: what do european forest managers want to know and what answers can science provide? Forest Ecology and Management 407, 106-115.
14. 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.
15. de-Dios-García, J., Manso, R., Calama, R., Fortin, M., Pardos, M.. 2018. A new multifactorial approach for studying intra-annual secondary growth dynamics in Mediterranean mixed forests: integrating biotic and abiotic interactions. Can. J. Forest Res. 48 (4), 333–344.
16. Deng, J., Wang, S., Ren, C., Zhang, W., Zhao, F., Li, X., Zhang, D., Han, X., Yang, G.. 2019. Nitrogen and phosphorus resorption in relation to nutrition limitation along the chronosequence of black locust (*Robinia pseudoacacia L.*) plantation. Forests 10(3), 261.
17. Dijkstra, F.A., Carrillo, Y., Aspinwall, M.J., Maier, C., Canarini, A., Tahaei, H., et al. 2016. Water, nitrogen and phosphorus use efficiencies of four tree species in response to variable water and nutrient supply. Plant and Soil 406(1-2), 187-199.
18. Dimitrakopoulos, P.G., Schmid, B.. 2004. Biodiversity effects increase linearly with biotope space. Ecol. Lett. 7(7), 574-583.
19. Drossler, L., Agestam, E., Bielak, K., Dudzinska, M., Koricheva, J., Liziniewicz, M., Lof, M., Mason, B., Pretzsch, H., Valkonen, S., Wellhausen, K.. 2018. Over- and underyielding in time and space in experiments with mixed stands of Scots Pine and Norway Spruce. Forests 9 (8), 18.

20. Ehleringer, J.R.. 1993. Variation in leaf carbon isotope discrimination in Encelia farinosa implications for growth competition, and drought survival. Oecologia 95(3), 340-346.
21. FAO. 2020. Global forest resources assessment. Food and Agriculture Organization of the United Natioms.
22. Farquhar, G.D., O'leary M.H, Berry, J.A.. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Functional Plant Biology 9(2), 121-137.
23. Farquhar, G.D., Richards, R.A.. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol. 11, 539-552.
24. Faustino, L.-I, Bulfe, N.M.L., Pinazo, M., Farquhar, G., Richards, R.. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Functional Plant Biology 11(6), 539-552.
25. Feller, I.C., Whigham, D.F., O'Neill, J.P., McKee, K.L.. 1999. Effects of nutrient enrichment on within-stand cycling in a mangrove forest. Ecology 2193-2205.
26. Forrester, D.I., Bauhus, J., Cowie, A.L.. 2005. 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.
27. Forrester, D.I., Bauhus, J., Cowie, A.L., Vanclay, J.K.. 2006. Mixed-species plantations of *Eucalyptus* with nitrogen-fixing trees: a review. For. Ecol. Manage. 233, 211-230.
28. Fu, B-J., Meng, Q-H., Qiu, Y.. 2004. Effects of land use on soil erosion and nitrogen loss in the hilly area of the Loess Plateau, China. Land Degradation & Development 15(1), 87-96.
29. Fu, L., Xu, Y., Xu, Z., Wu, B., Zhao, D., 2020. Tree water-use efficiency and growth dynamics in response to climatic and environmental changes in a temperate forest in Beijing. China Environ. Int. 134, 105209.
30. Garrish, V., Cernusak, L.A., Winter, K., Turner B.L.. 2010. Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, Ficus insipida. Journal of Experimental Botany 61, 3735-3748.
31. Gong, X., Chen, Q., Lin, S., Brueck, H., Dittert, K., Taube, F., Schnyder, H.. 2011. Tradeoffs between nitrogen- and water-use efficiency in dominant species of the semiarid steppe of InnerMongolia. Plant and Soil 340, 227-238.
32. Güsewell, S.. 2004. N:P ratios in terrestrial plants: Variation and functional significance. New Phytologist 164, 243-266.
33. Garrish, V., Cernusak, LA., Winter, K., et al. 2010. Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. Journal of Experimental Botany 61, 3735-3748.
34. Huang, Z., Liu, B., Davis, M., et al. 2015. Long-term nitrogen deposition linked to reduced water use efficiency in forests with low phosphorus availability. New Phytologist 210, 431-442.
35. Jennings, K.A., Guerrieri, R., Vadeboncoeur, M.A., Asbjornsen, H., 2016. Response of Quercus velutina growth and water use efficiency to climate variability and nitrogen fertilization in a temperate deciduous forest in the northeastern USA. Tree Physiol. 36, 428-443.
36. Lei, O.-Y., Lu, L.-W., Wang, C-L., Li, Y.-Q., Wang, J.-Y., Zhao, X.-H., Gao, L., Zhu, L.-W., Ni, G.-Y., Zhao, P.. 2022. A 14-year experiment emphasizes the important role of heat factors in regulating tree transpiration, growth, and water use efficiency of *Schima superba* in South China. Agricultural Water Management 1(273), 107902.
37. Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., van Ruijven, J., Weigelt, A., Wilsey, B.J., Zavaleta, E.S., Loreau, M.. 2011. High plant diversity is needed to maintain ecosystem services. Nature 477, 199-202.
38. Jia, G., Chen, L., Yu, X., Liu, Z., 2022. Soil water stress overrides the benefit of water-use efficiency from rising CO_2 and temperature in a cold semi-arid poplar plantation. Plant Cell Environ. 45, 1172-1186.
39. Katahata, S.I., Naramoto, M., Kakubari, Y., Mukai, Y.. 2007. Photosynthetic capacity and nitrogen partitioning in foliage of the evergreen shrub Daphniphyllum humile along a natural light gradient. Tree Physiology 27(2), 199-208.
40. Katul, G., Manzoni, S., Palmroth, S., Oren, R.. 2010. A stomatal optimization theory to describe the effects of atmospheric CO2 on leaf photosynthesis and transpiration. Annals of Botany 105(3), 431-442.
41. Khanna, M., Dhungana, B., Clifton-Brown, J.. 2008. Costs of producing *Miscanthus* and *switchgrass* for bioenergy in Illinois. Biomass and Bioenergy 32, 482-493.
42. Koerselman, W., Meuleman, A.F.M.. 1996. The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. J. Appl. Ecol. 33 (6), 1441.
43. Lebourgeois, F., Gomez, N., Pinto, P., Merian, P.. 2013. Mixed stands reduce Abies alba tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. For. Ecol. Manage. 303, 61-71.
44. Liu, Y.-B, Xiao, J.-F, Ju, W.-M, Zhou, Y.-L., Wang, S.-Q., Wu, X.-C.. 2015. Water use efficiency of China's terrestrial ecosystems and responses to drought. Scientific Reports 5, 13799.
45. Luo, Y., Su, B., Currie W.S., Dukes, J.S., Finzi, A., Hartwig, U., Hungate, B.A., Mcmutrie, R.E., Oren, R., Parton, W.,J., Pataki, D.E., Shaw, M.R., .Zak, D.R., Field, C.B.. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54(8), 731-739.
46. Meier, S., Morales, A., López-Olivari, R.A., Matus., I., Aponte, H., Campos, P.S., Khan, N., Cartes P., Meriño-Gergichevich, C., Castillo, D., Seguel, A... 2022. Synergistic role between phosphorus and water use efficiency in spring wheat genotypes. Agricultural Water Management 263, 107481.
47. Cregger, M.A., McDowell, N.G., Fangle., R.E., Pockman, W.T., Classen, A.T.. 2014. The impact of precipitation change on nitrogen cycling in a semi-arid ecosystem. Functional Ecology 28, 1534-1544.
48. 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.

49. Nelson, D., Sommers, L.E.. 1982. Total carbon, organic carbon, and organic matter. In: 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.
50. Nygren, P., Leblanc, H.A.. 2015. Dinitrogen fixation by legume shade trees and direct transfer of fixed N to associated cacao in a tropical agroforestry system. Tree Physiol. 35, 134-147.
51. Patterson, T.B., Guy, R.D., Dang, Q.-L.. 1997. Whole- plant nitrogen- and water- relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal spruce species. Oecologia 110, 160-168.
52. Penuelas, J., Canadell, J.G., Ogaya, R.. 2011. Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. Global Ecology and Biogeography 20(4), 597-608.
53. Pretzsch, H., Schutze, G., Uhl, E.. 2013. Resistance of European tree species to drought stress in mixed versus monocultures forests: evidence of stress release by inter-specific facilitation. Plant Biology 15(3), 483-495.
54. Qiu, X.-C., Wang, H.-B., Peng, D.-L., Liu, X., Yang, F., Li, Z., Cheng, S.. 2020. Thinning drives C:N:P stoichiometry and nutrient resorption in larix principis-rupprechtii plantations in North China. Forest Ecology and Management 462, 117984.
55. 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 and Soil 332, 207-217.
56. Ripullone, F., Lauteri, M., Grassi, G., Amato, M., Borghetti, M.. 2004. *Pseudotsuga menziesii* and *Populus* x *euroamericana*; a comparison of three approaches to determine water-use efficiency. Tree Physiol. 24, 671-679.
57. Sardans, J., Peñuelas, J.. 2012. The role of plants in the effects of global change on nutrient availability and stoichiometry in the plant-soil system. Plant Physiology 160, 1741-1761.
58. Shan, C., Liang, Z., Hao, W.. 2002. Review on growth of locust and soil water in Loess Plateau. Acta Botanica Boreali-Occidentalia Sinica 23, 1341-1346.
59. Stubbs, W.J., Wilson, J.B.. 2004. Evidence for limiting similarity in a sand dune community. J. Ecol. 92 (4), 557-567.
60. Su, B.-Q., Shangguan, Z.-P.. 2020. Patterns and driving factors of water and nitrogen use efficiency in Robinia pseudoacacia L. on the Loess Plateau in China. Catena 195, 104790.
61. Tanaka-Oda, A., Kenzo, T., Koretsune, S., Sasaki, H., Fukuda, K., 2010. Ontogenetic changes in water-use efficiency ($\delta^{13}C$) and leaf traits differ among tree species growing in a semiarid region of the Loess Plateau, China, Forest Ecology and Management 259(5), 953-957.
62. Taylor, B.N., Chazdon, R.L., Bachelot, B., Menge, D.N.L.. 2017. Nitrogen-fixing trees inhibit growth of regenerating Costa Rican rainforests. Proc. Natl. Acad. Sci. U. S. A. 114(33), 8817-8822.
63. Turner, J., Lambert, M.J.. 2014. Analysis of nutrient use efficiency (NUE) in Eucalyptus pilularisforests. Australian Journal of Botany 62, 558-569.
64. Vitousek, P.M. 1982. Nutrient cycling and nutrient use efficiency. Am Nat. 119, 553-572.
65. Vitousek, P.M., Porder, S., Houlton, B.Z., et al. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications 20, 5-15.
66. Voigtlaender, M., Laclau, J.-P., Gonçalves, J.L.M., Piccolo, M.C., Moreira, M.Z., Nouvellon, Y., Ranger, J.-P., Bouillet, J.-P.. 2012. Introducing Acacia mangium trees in Eucalyptus grandis plantations: consequences for soil organic matter stocks and nitrogen mineralization. Plant and Soil 352, 99-111.
67. Wang, J., Fu, B.-J., Jiao, L. , Lu, Nan., Li, J.-Y., Chen W.-L., Wang, L.-X.. 2021. Age-related water use characteristics of Robinia pseudoacacia on the Loess Plateau. Agricultural and Forest Meteorology 301-302.
68. Warren, C.R., McGrath, J.F., Adams, M.A.. 2005. Differential effects of N, P and K on photosynthesis and partitioning of N in Pinus pinaster needles. Ann For Sci 62, 1-8.
69. 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.
70. Wu, J.-E., Liu, W.-J., Chen, C.-F.. 2017. How do plants share water sources in a rubber-tea agroforestry system during the pronounced dry season?. Agriculture Ecosystems & Environment 236, 69-77.
71. Yan, W.-M., Zhong, Y.Q.W., Zheng, S-X., Shangguan, Z-P.. 2016. Linking plant leaf nutrients/stoichiometry to water use efficiency on the Loess Plateau in China. Ecological Engineering 87, 124-131.
72. Yan, T., Wang, L-Y., Zhong, T-Y., Fu, C.. 2022. Decadal nitrogen addition increases divergence in intrinsic water-use efficiency between sapling and mature larch plantations. Forest Ecology and Management 523, 120494.
73. Zhao, H.-X., Duan, B.-L., Lei, Y.-B.. 2015. Causes for the unimodal pattern of leaf carbon isotope composition in Abies faxoniana trees growing in a natural forest along an altitudinal gradient. J. Mountain Sci. 12, 39-48.
74. Zhou, Y.-C., Cheng, X.-L., Fan, J.-W., Harris, W.. 2016. Relationships between foliar carbon isotope composition and elements of C_3 species in grasslands of inner mongolia, China. Plant Ecology 217(7), 883-897.
75. Zou, X.-D., Binkley, D., Caldwell, B.A.. 1995. Effects of dinitrogen-fixing trees on phosphorus biogeochemical cycling in contrasting forests. Soil Sci. Soc. Am. J. 59, 1452–1458.

Tables

Table 1. Information of sampling sites.

Plantation type	Main tree species	Plantation	No.	Longitude (N)	Latitude (E)	Altitude (m)	Gradient (°)	Slope	Soil	Understory plant species
Mixture	*R. pseudoacacia, A. davidiana*	RPAD	1	109°13'42.56"	36°35'52.03"	1196	20.4	South-west	Loess	*Viola dactyloides, Artemisia gmelinii*
	R. pseudoacacia, A. davidiana	RPAD	2	109°13'27.27"	36°35'29.35"	1191	29.2	South-west	Loess	*Viola dactyloides, Artemisia gmelinii*
	R. pseudoacacia, A. davidiana	RPAD	3	109°13'12.22"	36°35'30.82"	1199	22.6	South-west	Loess	*Viola dactyloides, Artemisia gmelinii*
	R. pseudoacacia, A. sibirica	RPAS	1	109°16'7.25"	36°35'34.06"	1192	22.3	South-west	Loess	*Viola dactyloides, Artemisia gmelinii*
	R. pseudoacacia, A. sibirica	RPAS	2	109°13'45.88"	36°35'30.27"	1191	23.9	South-west	Loess	*Viola dactyloides, Artemisia gmelinii*
	R. pseudoacacia, A. sibirica	RPAS	3	109°13'36.87"	36°35'50.74"	1203	20.4	South-west	Loess	*Viola dactyloides, Artemisia gmelinii*
Monoculture	*R. pseudoacacia*	RP	1	109°13'39.72"	36°35'50.85"	1203	21.4	South-west	Loess	*Artemisia gmelinii, Stipa bungeana*
	R. pseudoacacia	RP	2	109°13'56.46"	36°35'24.88"	1200	21.7	South-west	Loess	*Artemisia gmelinii, Stipa bungeana*
	R. pseudoacacia	RP	3	109°13'19.84"	36°35'29.70"	1197	14.0	South-west	Loess	*Artemisia gmelinii, Stipa bungeana*
	A. davidiana	AD	1	109°13'36.79"	36°35'50.23"	1217	28.8	South-west	Loess	*Artemisia gmelinii, Rubia cordifolia*
	A. davidiana	AD	2	109°13'43.41"	36°35'49.31"	1202	25.0	South-west	Loess	*Artemisia gmelinii, Rubia cordifolia*
	A. davidiana	AD	3	109°13'39.86"	36°35'48.03"	1201	21.0	South-west	Loess	*Artemisia gmelinii, Rubia cordifolia*
	A. sibirica	AS	1	109°13'50.28"	36°35'57.59"	1160	24.0	South-west	Loess	*Artemisia gmelinii, Rubia cordifolia*
	A. sibirica	AS	2	109°13'56.90"	36°35'48.96"	1222	18.6	South-west	Loess	*Artemisia gmelinii, Rubia cordifolia*
	A. sibirica	AS	3	109°16'31.85"	36°36'40.02"	1224	17.3	South-west	Loess	*Artemisia gmelinii, Rubia cordifolia*

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

Table 2. Measurement and calculation methods of plant functional traits.

Plant functional traits	Initials	Measurement and calculation	Unit
Fresh leaf weight	FW	Electronic balance (accuracy 0.001 g)	g
Dry leaf weight	DW	Electronic balance	g
Leaf thickness	LT	Digital vernier caliper	mm
Leaf area	LA	Epson Perfection V850 Pro Scanner, ImageJ software	cm^{-2}
Leaf organic carbon content per unit mass	MLCC	Potassium dichromate — external heating method	$g{\cdot}kg^{-1}$
Leaf nitrogen content per unit mass	N_{mass}	Kjeldahl method (Kjektec System 2300 Distilling Unit, Foss, Sweden)	$g{\cdot}kg^{-1}$
Leaf phosphorus content per unit mass	P_{mass}	Molybdenum antimony anti-colorimetric method (U-2800 Spectrophotometer, Shanghai, China)	$g{\cdot}m^{-2}$
Specific leaf area	SLA	SLA=DW/LA	$g{\cdot}cm^{-2}$
Leaf dry matter content	LDMC	LDMC=DW/FW	g
Leaf tissue density	LD	LD=DW/(LT • LA)	$g{\cdot}cm^{-3}$
Leaf nitrogen content per unit area	N_{area}	ALNC=MLNC • 10/SLA	$g{\cdot}m^{-2}$
Leaf phosphorus content per unit area	P_{area}	ALPC=MLPC • 10/SLA	$g{\cdot}m^{-2}$

Table 3. Tree structure and leaf functional traits.

Tree species	Plantation	Tree structure					Leaf functional traits								
		SD	DBH	AH	CA	HB	LT	LA	SLA	LDMC	LD	N_{area}	N_{mass}	Par_{ea}	P_{mass}
R.pseudoacacia	RPAD	1192±22a	9.30±0.22b	8.52±0.22b	4.71±0.38c	3.33±0.14a	0.10±0.01e	12.62±0.47d	25.38±1.41b	0.33±0.01c	0.37±0.02c	11.02±0.73b	27.76±0.23a	0.49±0.03b	1.23±0.01ab
	RPAS	1025±80a	9.21±0.24b	8.44±0.18b	6.24±0.35b	3.13±0.06a	0.11±0.00e	17.10±0.41b	25.67±0.86b	0.32±0.00c	0.38±0.00c	10.38±0.40b	26.56±0.20b	0.50±0.02b	1.29±0.03a
	RP	1083±42a	11.55±0.96 a	9.54±0.41a	6.52±0.50b	3.23±0.36a	0.09±0.00f	11.53±0.30d	35.37±1.22a	0.28±0.01c	0.32±0.01c	7.98±0.20c	28.18±0.42a	0.35±0.02c	1.22±0.03ab
A. davidiana	RPAD	325±14c	5.16±0.15c	4.11±0.05d	6.19±0.08b	0.92±0.04b	0.14±0.00d	16.50±0.58b	22.39±0.39b	0.30±0.00c	0.32±0.01c	11.24±0.28b	25.14±0.24c	0.52±0.01b	1.17±0.01b
	AD	733 ±33b	6.32±0.12c	3.58±0.12d	4.88±0.05c	0.34±0.01b	0.16±0.00c	14.02±0.40c	11.59±0.14d	0.48±0.03a	0.53±0.02a	15.39±0.2a	17.83±0.09d	0.78±0.03a	0.90±0.03c
A. sibirica	RPAS	308±55c	9.75±0.12b	5.62±0.12c	7.42±0.07a	0.60±0.02b	0.23±0.00a	18.94±0.23a	14.90±1.08c	0.33±0.02c	0.32±0.01c	17.04±1.15a	25.14±0.24c	0.79±0.06a	1.17±0.01b
	AS	700±58b	10.09±0.77ab	3.46±0.05d	4.26±0.21c	0.37±0.01b	0.20±0.01b	18.87±0.42a	11.89±0.08d	0.39±0.00b	0.43±0.02 b	11.08±0.08b	13.18±0.10e	0.69±0.02a	0.83±0.03d
One-way ANOVA	*F*-value	54.213	21.087	166.823	15.930	92.860	123.956	51.262	95.792	27.641	29.661	30.864	559.974	30.143	66.396
	P-value	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

Note: SD, stand density (• 10^3 number hm^{-2}); DBH, diameter at breast height (cm); AH, average tree height (m); CA, crown area (m); HB, height under the stem (m); LT, leaf thickness (mm); LA, leaf area (cm^{-2}); SLA, specific leaf area ($g \cdot cm^{-2}$); LDMC, leaf dry matter content (g); LD, leaf tissue density ($g \cdot cm^{-3}$); N_{area}, leaf nitrogen content per unit area ($g \cdot m^{-2}$); N_{mass}, leaf nitrogen content per mass ($g \cdot kg^{-1}$); P_{area}, leaf phosphorus content per unit area ($g \cdot m^{-2}$); P_{mass}, leaf phosphorus content per mass ($g \cdot kg^{-1}$).

Table 4. Soil physical and chemical properties.

Afforestation pattern	Plantation	Soil physical properties						Soil chemical properties	
		BD	SWC	EC	SA	SI	CL	pH	SOC
Mixture	RPAD	1.25±0.05a	11.13±0.63a	107.57±0.98a	16.40±0.10b	51.66±1.55a	31.94±1.49b	8.58±0.12a	6.02±0.12c
	RPAS	1.25±0.04a	9.91±0.08b	107.33±1.11a	15.17±0.17bc	54.46±0.71a	30.37±0.54b	8.52±0.06a	7.09±0.28b
Monoculture	RP	1.24±0.05a	9.44±0.06b	105.50±0.60a	14.79±0.56c	55.05±1.10a	30.16±0.56b	8.66±0.01a	5.78±0.26c
	AD	1.19±0.05b	10.54±0.08ab	82.30±0.76c	15.66±0.12bc	54.07±0.35a	30.27±0.32b	8.72±0.03a	3.94±0.09d
	AS	1.27±0.05a	7.91±0.19c	87.95±0.81b	17.69±0.49a	48.40±0.67b	33.91±0.19a	8.72±0.03a	8.59±0.11a
One-way ANOVA	*F*-value	54.213	21.087	166.823	15.930	92.866	123.956	51.262	95.792
	P-value	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

Note: BD, soil bulk density ($g \cdot cm^{-3}$); SWC, soil water content (%); EC, soil conductivity ($mS \cdot cm^{-1}$); SA, soil sand content (%); SI, soil silt content (%); CL, soil clay content (%); pH, soil pH; SOC, soil organic carbon ($g \cdot kg^{-1}$); TN, soil total nitrogen ($g \cdot kg^{-1}$); TP, soil total phosphorus ($g \cdot kg^{-1}$); AN, soil-available nitrogen ($mg \cdot kg^{-1}$); AP, soil-available phosphorus ($mg \cdot kg^{-1}$).

Table 5. Effect of tree species (TS), plantation type (PT), and their interaction (TS×PT) on WUE, NUE, PUE, and N:P (repeated measures ANOVA).

Factors	df	WUE		NUE		PUE		N:P	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
TS	2	11.348	0.001**	897.978	0.000**	136.023	0.000**	86.209	0.000**
PT	1	5.613	0.032*	407.727	0.000**	167.251	0.000**	0.485	0.497
TS×PT	2	0.255	0.778	131.177	0.000**	37.847	0.000**	5.014	0.022*

Table 6. Partial correlation coefficients of WUE, NUE, PUE, and N:P ratio with their potential driving factors.

Variable	Factors	WUE		NUE		PUE		N:P	
		Coef.	Sig.	Coef.	Sig.	Coef.	Sig.	Coef.	Sig.
Tree structure	SD	-0.641	0.006**	0.525	0.030*	0.763	0.000**	0.103	0.694
	DBH	0.442	0.076	0.909	0.000**	0.281	0.274	-0.766	0.000**
	AH	0.371	0.143	-0.734	0.001	-0.445	0.073	0.490	0.046*
	CA	-0.508	0.031*	0.222	0.392	0.237	0.360	-0.199	0.445
	HB	0.636	0.005 **	-0.284	0.270	-0.668	0.003**	-0.277	0.283
Leaf characteristic	LT	0.234	0.441	0.828	0.000**	0.198	0.516	-0.645	0.017*
	LA	-0.351	0.240	0.314	0.295	-0.322	0.284	-0.293	0.331
	SLA	-0.641	0.018	0.704	0.007**	0.495	0.085	-0.337	0.260
	LDMC	-0.095	0.758	0.360	0.227	-0.104	0.736	-0.212	0.486
	LD	-0.041	0.894	0.092	0.766	0.284	0.347	0.014	0.964
	ALNC	-0.734	0.004 **	-0.553	0.050**	0.564	0.044*	0.330	0.271
	MLNC	0.654	0.015	-0.102	0.740	-0.588	0.035*	0.042	0.892
	ALPC	0.692	0.009 **	0.631	0.021*	-0.468	0.107	-0.339	0.257
	MLPC	-0.486	0.092	-0.387	0.191	0.164	0.592	-0.169	0.582
Soil physical properties	BD	0.128	0.637	0.302	0.256	0.156	0.563	-0.335	0.177
	SWC	-0.217	0.419	-0.277	0.298	0.175	0.517	0.267	0.318
	EC	0.458	0.074	-0.550	0.027*	-0.839	0.000**	0.170	0.530
	SA	0.138	0.610	0.228	0.396	-0.046	0.867	-0.285	0.284
	SI	0.136	0.615	0.227	0.397	-0.045	0.869	-0.285	0.285
	CI	0.136	0.617	0.227	0.397	-0.044	0.872	-0.285	0.285
Soil chenical properties	pH	-0.040	0.883	0.119	0.660	0.171	0.527	0.051	0.851
	SOC	0.196	0.467	0.134	0.621	-0.030	0.913	-0.313	0.238
	TN	0.082	0.762	-0.019	0.945	-0.244	0.362	-0.187	0.488
	TP	0.104	0.701	-0.534	0.033*	-0.587	0.017*	0.309	0.243
	AN	-0.145	0.592	0.170	0.529	0.299	0.260	0.149	0.582
	AP	0.409	0.116	-0.620	0.010*	-0.610	0.012*	0.598	0.014*

Figures

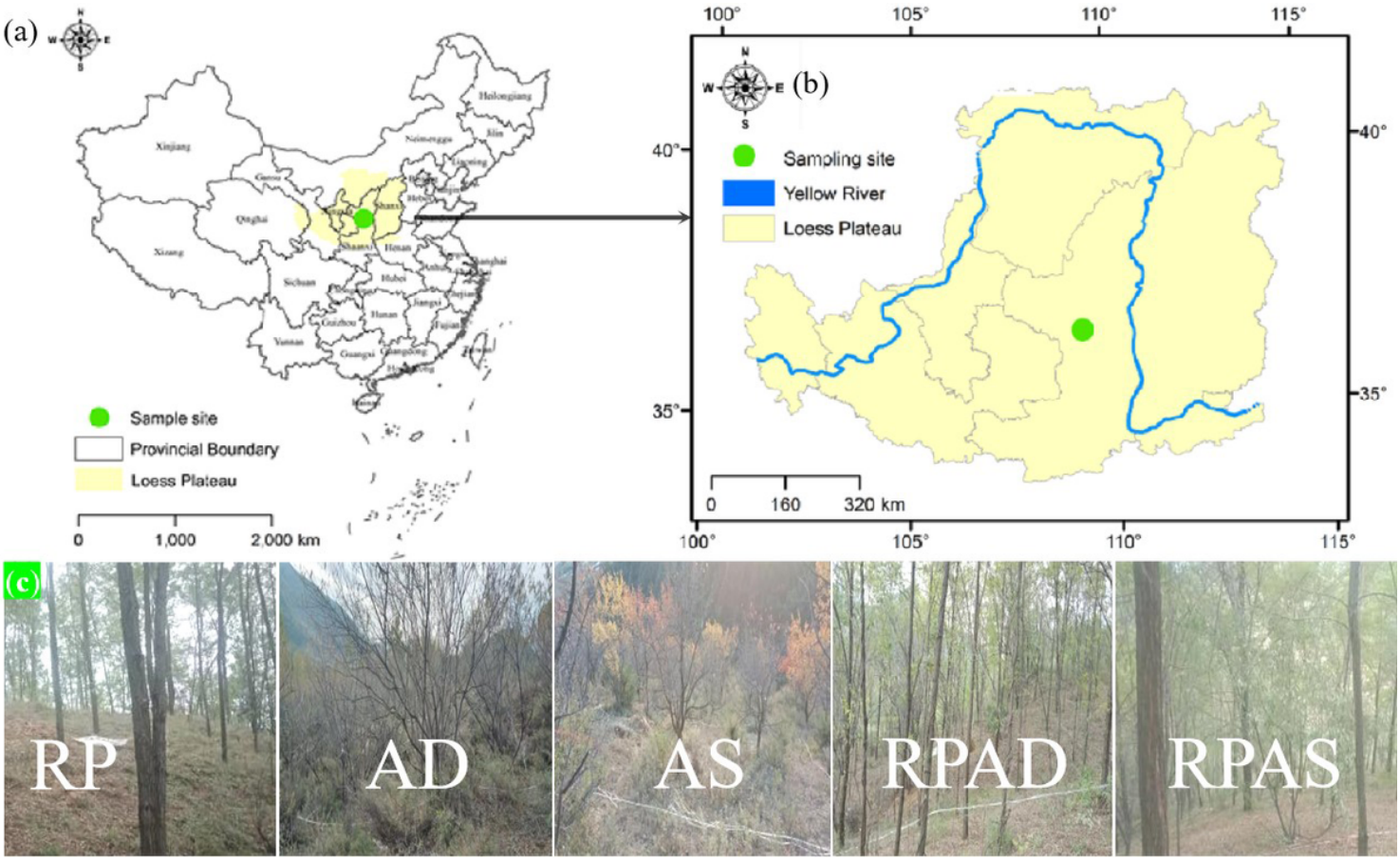


Figure 1

Mixed and monoculture stands (C) are located on the Loess plateau (B) of China (A). RPAD and RPAS represent mixed stands of *R. pseudoacacia* with *A. davidiana* and *R. pseudoacacia* with *A. sibirica*, respectively. RP, AD, and AS represent monoculture stands of *R. pseudoacacia*, *A. davidiana*, and *A. sibirica*, respectively.

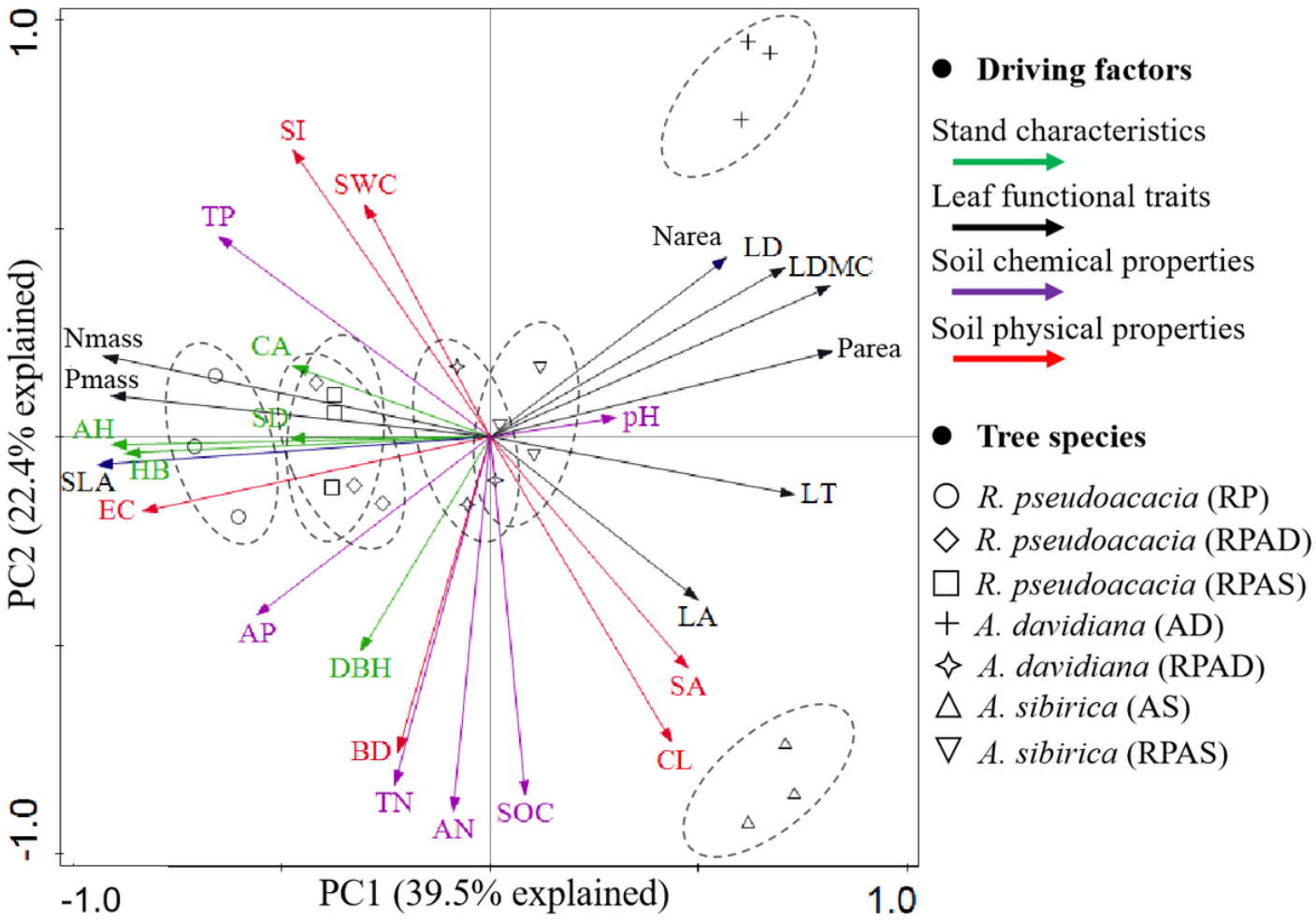


Figure 2

Principal component analysis of variables. SD, stand density; DBH, diameter at breast height; AH, average tree height; CA, crown area; HB, height under the stem; LT, leaf thickness; LA, leaf area; SLA, specific leaf area; LDMC, leaf dry matter content; LD, leaf tissue density; N_{area}, leaf nitrogen content per unit area; N_{mass}, leaf nitrogen content per mass; P_{area}, leaf phosphorus content per unit area; P_{mass}, leaf phosphorus content per mass. BD, soil bulk density; SWC, soil water content; EC, soil conductivity; SA, soil sand content; SI, soil silt content; CL, soil clay content; pH, soil pH; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AN, soil available nitrogen; AP, soil available phosphorus.

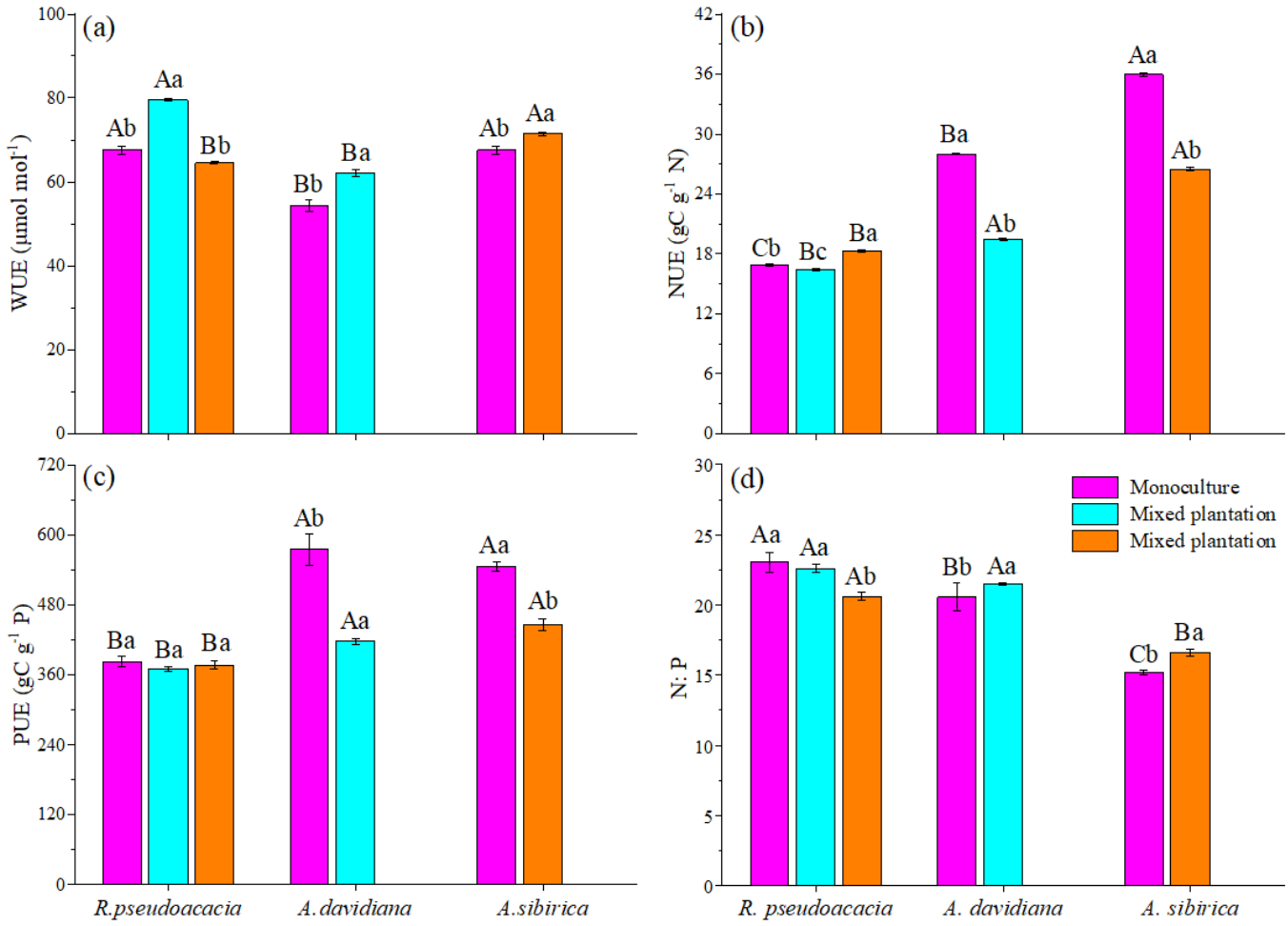


Figure 3

Average leaf (a) WUE, (b) NUE, (c) PUE, and (d) N:P ratio. WUE, NUE,and PUE represent water, nitrogen, and phosphorus use efficiencies, respectively. Different uppercase letters indicate significant differences among different tree species for the same plantation type (P< 0.05), while different lowercase letters above the bars indicate significant differences among the same tree species for different plantation types (P < 0.05).

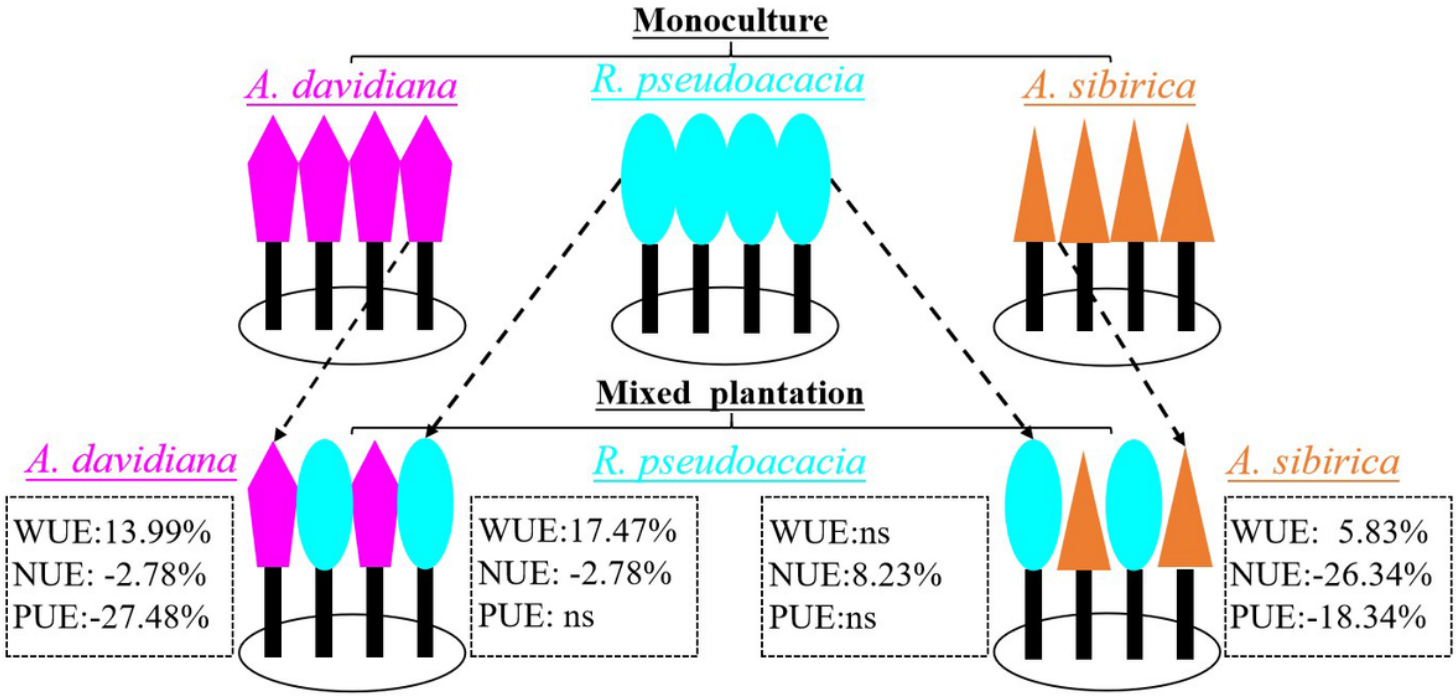


Figure 4

Changes in WUE, NUE, and PUE of three species when transitioning from monoculture to mixed stand. WUE, NUE,and PUE represent water, nitrogen, and phosphorus use efficiencies, respectively.

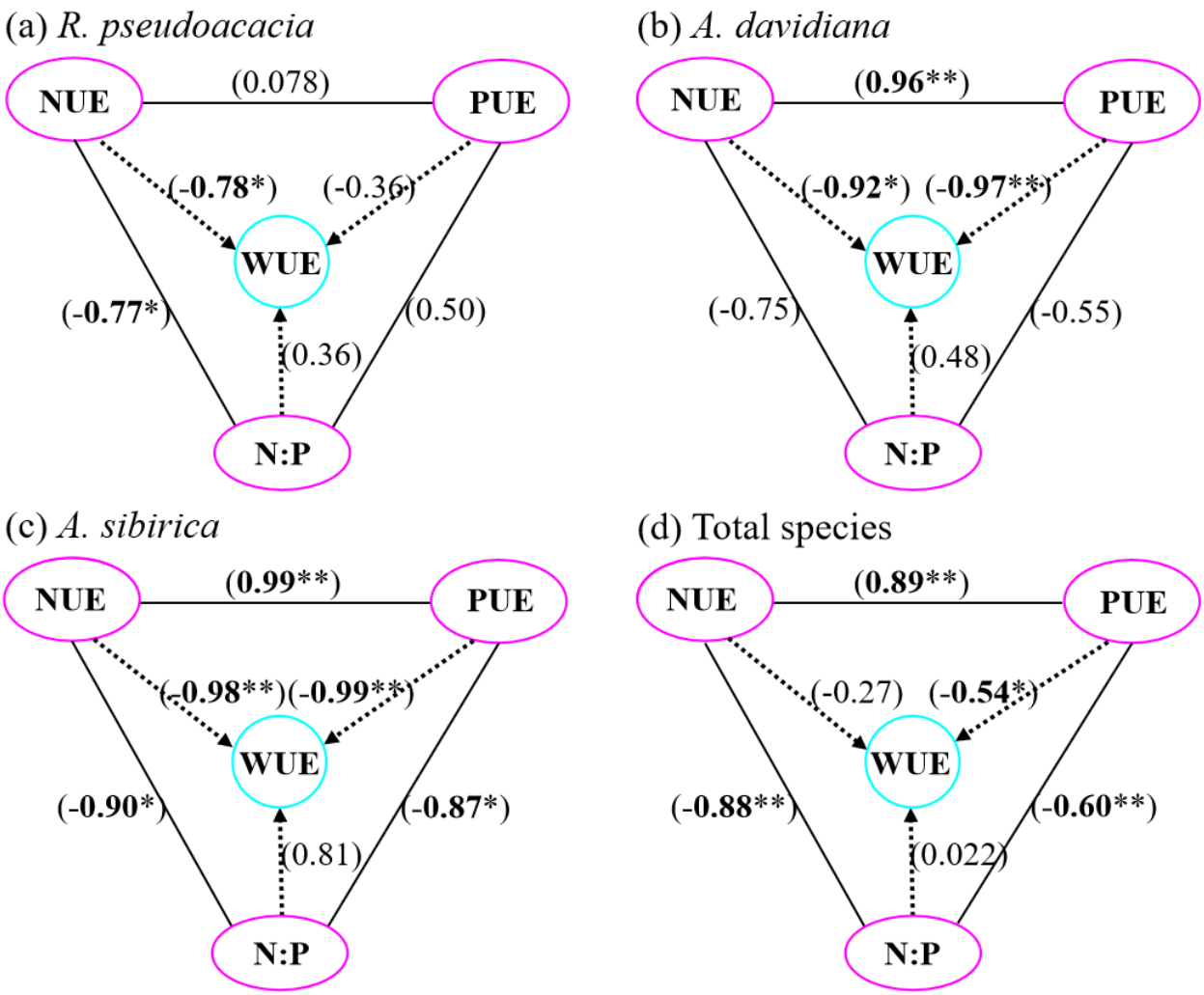


Figure 5

Pearson correlations among WUE, NUE, PUE, and N:P ratio of (a) *R. pseudoacacia*, (b) *A. davidiana*, (c) *A. sibirica*, and (d) total species in monoculture and mixed stands. WUE, NUE, and PUE represent water, nitrogen, and phosphorus use efficiencies, respectively.

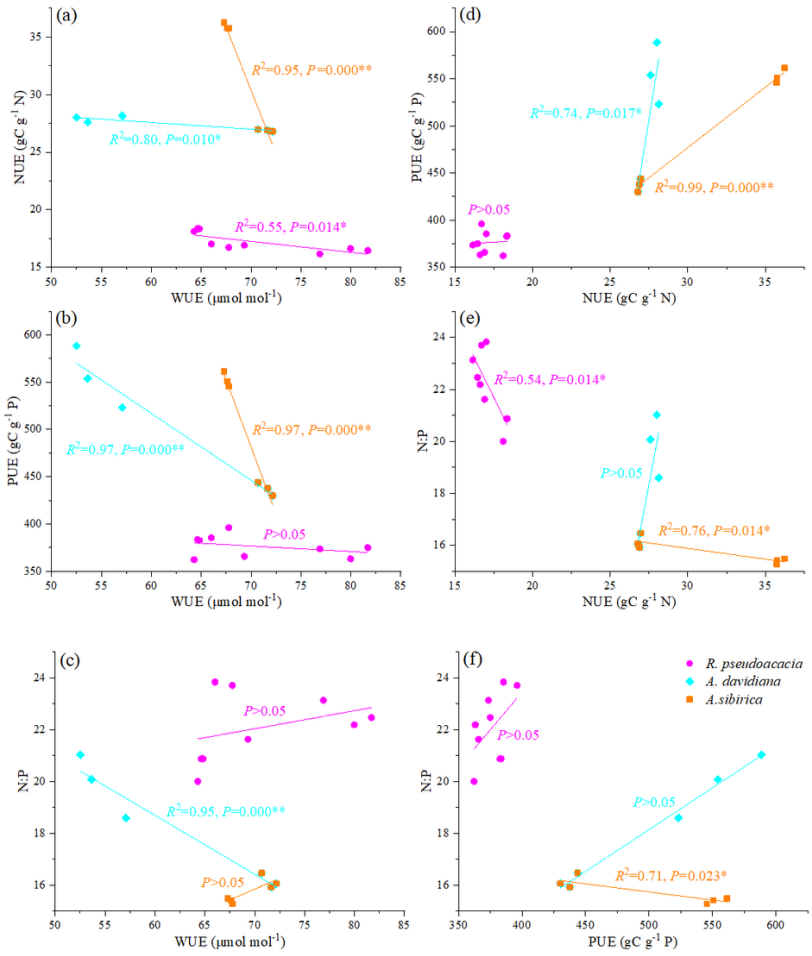


Figure 6

Linear relationship between WUE, NUE, PUE, and N:P ratio of different tree species; subfigures (a), (b), (c), (d), (e), and (f) show the relationship between WUE and NUE, WUE and PUE, WUE and N:P ratio, NUE and PUE, NUE and N:P ratio, and PUE and N:P ratio, respectively.

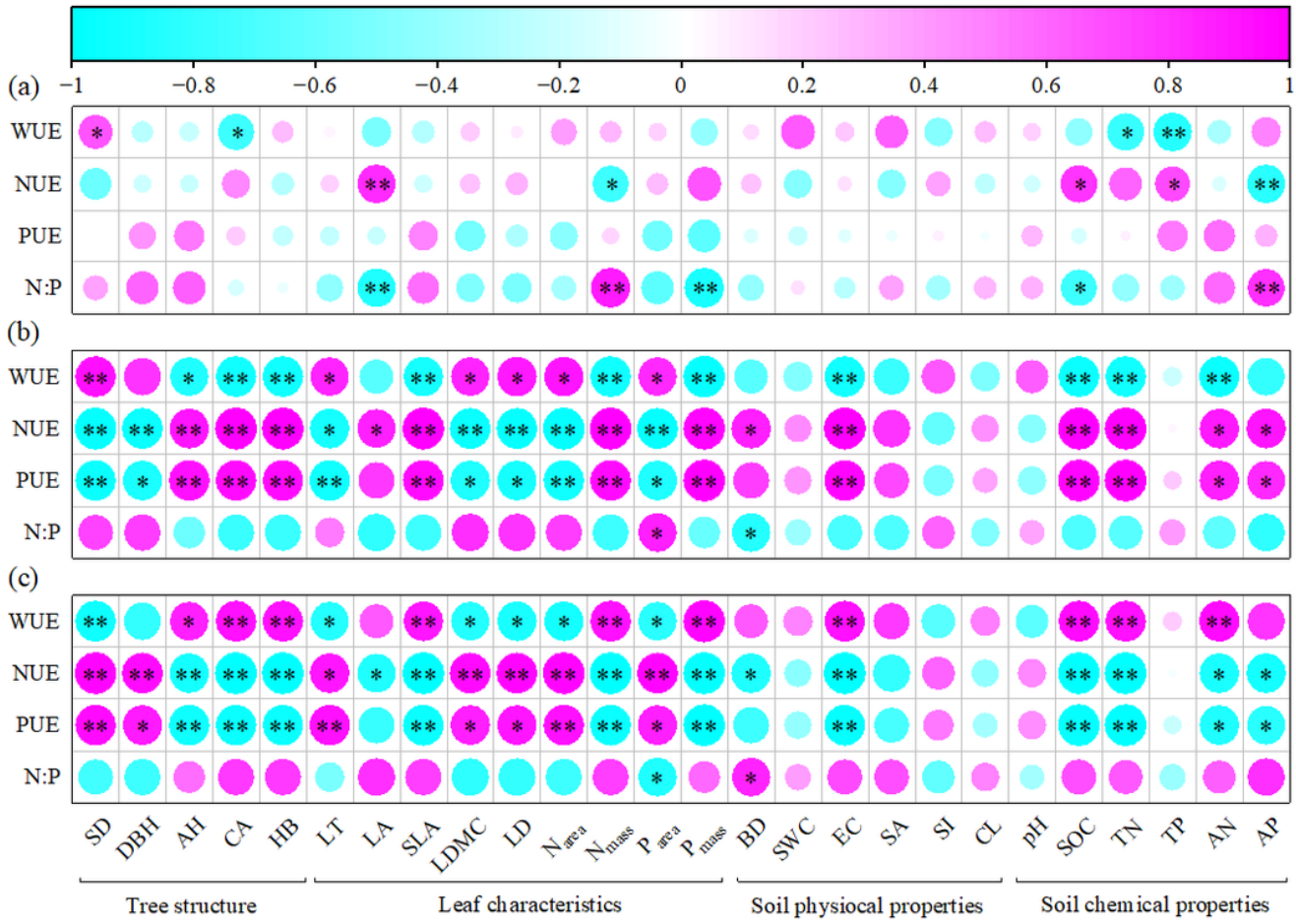


Figure 7

Correlation matrix of WUE, NUE, PUE, and N:P ratio of (a) *R. pseudoacacia*, (b) *A. davidiana*, and (c) *A. sibirica* with tree structure and leaf functional traits and soil properties in monocultureand mixed stands.

traits and soil properties in monoculture and mixed plantation.